NUCLEAR AND CELL DIVISION IN ALLIUM CEPA AS INFLUENCED BY SLOW NEUTRONS AND X-RAYS[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 622

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Introduction

Abnormal behavior in nuclear and cell division can be induced by many different agencies: marked temperature changes, regeneration of tissue, genetic factors, chromosomal incompatibilities in species hybrids, narcotics and chemicals, disease and injury, osmotic changes, ultraviolet light, X-ray treatment, neutron bombardment, and by internal radiation through use of radioactive isotopes (**1, 4, 7, 8, 10, 11, 14, 15, 20, 21, 22, 25, 26**). Regardless of the agent used, the resulting irregularities are similar in most respects.

Structural changes in chromosomes also can be induced by temperature changes, chemical agents, genetic factors, aging of seed, and both ionizing and non-ionizing radiations (**1, 4, 6, 14, 20, 21, 22, 23, 25, 27**). CATCHESIDE (**1**), NICHOLS (**19**), and SAX (**22**) have indicated that such induced changes are similar to those occurring naturally or spontaneously.

Cytological effects of irradiation by X-rays and fast neutrons have been recently comprehensively reviewed (**1, 14, 15**). Plant materials so studied have been limited primarily to *Tradescantia* microspores and to root tips of onion, bean, pea, and tomato seedlings.

Among earlier investigations of radiobiological effects of slow neutrons were reports of specific cell destruction induced in neoplastic tissues (**12, 29**). Later studies to determine survival doses for mice were reported by ZIRKLE (**30**) and HENSHAW *et al.* (**9**). Most recently, the cytological effects on *Tradescantia* chromosomes of slow neutrons from the Oak Ridge pile have been reported by CONGER and GILES (**3**). Preliminary results indicated that frequencies of both one- and two-hit aberrations increased linearly with increases in dose of slow neutrons. CONGER (**2**) found that 99% of the effects induced result from the $H^1(n, \gamma)H^2$, $N^{14}(n, p)C^{14}$, and $B^{10}(n, \alpha)Li^7$ nuclear reactions.

With the exception of the recent report by FROLIK and MORRIS (**5**) on effects of irradiation of maize pollen in a nuclear reactor, no published data of radiobiological effects of slow neutrons from the Argonne heavy-water pile have been found in the literature.

Reports by many workers on types of chromosomal aberrations, their frequencies, and relationships to dose as well as to type of radiation in various plant materials suggested that a cytological comparison of the effects of X-rays and of slow neutrons on onion might prove of interest.

Material and methods

Sets of *Allium cepa*, variety Ebenezer, were obtained and irradiated during the first week of May, 1949, by Dr. N. J.

[1] This work was supported in part by grants from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago and from the Atomic Energy Commission.

SCULLY of the Division of Biology of the Argonne National Laboratory.

For the X-irradiated material, the following radiation factors were employed: 200 kv; 0.275 A; target subject distance, 35 inches; no added filtration; size of field, 4 inches; 25 r/minute as measured in air using a Victoreen thimble r-meter at the subject position on the table. The table revolved throughout all irradiation periods to insure uniformity of dose. The effective wave length of 0.275 A was based on a half-value layer of aluminum of 5.5 mm. With the constant dose rate employed, exposure periods of 4, 40, and 400 minutes resulted in total doses of 100 r, 1000 r, and 10,000 r, respectively.

For the slow-neutron treatment the material was placed in the thermal neutron column of the Argonne heavy-water pile, in a position as close to the pile proper as was possible. The pile was operated at 250 kw. This yielded 5.8×10^{10} neutrons/sq cm/sec at the position of the bulbs. Exposure periods of 2, 4, 8, and 16 minutes were given. Since a linear relationship exists, the dose should be proportional to exposure time. The pile was started from zero power for any one exposure period, and the time required to reach desired power (250 kw) was approximately 1.5–2.5 minutes. However, the time required to bring the pile up to operation power was considered. For example, in the 2-minute exposure, 2 minutes were required to bring the power from zero to 250 kw (linear for this time period); therefore, this was equal to 1 minute at 250 kw, and 1 more minute was allowed while at 250 kw to give a total of 2 minutes at this power.

It was not possible in these experiments to provide shielding of the materials in such a way that a pure source of any one type of radiation, i.e., slow neutrons, could be obtained. Observed effects must be assigned chiefly to both slow neutrons and gamma rays. The percentage of each of these is not known. Hence dose rate can only be referred to as the average flux of neutrons at subject position, i.e., 5.8×10^{10} neutrons/sq cm/sec. The total slow-neutron dose was obtained by multiplying the average flux of neutrons by the number of seconds of exposure.

Bulbs from all treatments were potted on May 10, 1949, 3 days after the X-irradiation treatment and 5 days after the slow-neutron treatment. Root-tip collections were made 48, 52, and 96 hours later. To obtain a maximum number of division figures, collections were made at noon for 48- and 96-hour materials.

Several root tips from each series were treated in 0.2% solutions of colchicine for 30 minutes, fixed in alcohol-acetic, and propiono-carmine squash preparations of such materials were examined for metaphase configurations. The majority of the root tips, however, were fixed in SAX's modification of Navashin's solution, dehydrated according to the tertiary butyl alcohol schedule, imbedded in paraffin, and sectioned at 12 μ. Both gentian violet-iodine and aqueous haematoxylin techniques were used in staining.

Analysis of division figures in the sectioned material was based largely on anaphase stages. Over 13,000 figures were studied. Aberration frequency was calculated from the total number of chromosomal aberrations (dicentrics, centric rings, and fragments) scored in from one hundred to five hundred cells on from six to ten slides for each dosage. Sampling errors were calculated from the formula given by THODAY (**27**):

$$\text{per cent sampling error} = \frac{100\sqrt{n}}{T}.$$

where n is the total number of aberrations scored, and T is the total number of cells observed. In the present experiments percentages of error were within 10% of the mean, save for the highest dosage with slow neutrons, in which case it was within 15% of the mean.

For the study of inhibition of mitosis, counts were made of all cells in a median longisection of each root tip for each treatment. A total of 340,000 cells was surveyed. In addition to scoring energic and actively dividing cells for materials treated with X-ray or slow neutrons, in the latter series counts were also made of the cells in each phase of the mitotic cycle.

Observations

Gross effects

Deleterious effects from both types of treatment became evident soon after planting the bulbs. For the highest dosages only about half of twenty or more bulbs used from each type of treatment had produced roots at the 48-hour collection. For the lower doses with X-ray, no apparent delay in sprouting was noted.

Figures 1 and 2, taken 3 weeks after planting, indicate general retardation of shoot development. In comparison with controls for each series, the lowest dosage of slow neutrons was as severely damaging to the bulbs as the highest X-ray dosage. The severity of the slow-neutron treatment is particularly marked when the gradual slope of retarded shoot development in the X-ray series is compared with the sudden and precipitous drop in the slow-neutron series. It was found that all the slow-neutron treatments were lethal; all plants in these series died, as did also all plants given the highest dosage of X-rays.

Bulbs from the same lots as described above were planted in the greenhouse and in the field at Argonne by Dr. Scully. Data on twenty-five bulbs for each dosage level over a 10-week period showed that all treated plants grown in the field failed to survive. At the 10-week survey there was an 8% survival of greenhouse-grown bulbs which had been exposed for 16 minutes to slow neutrons. By 15 weeks, however, all plants treated with slow neutrons had died.

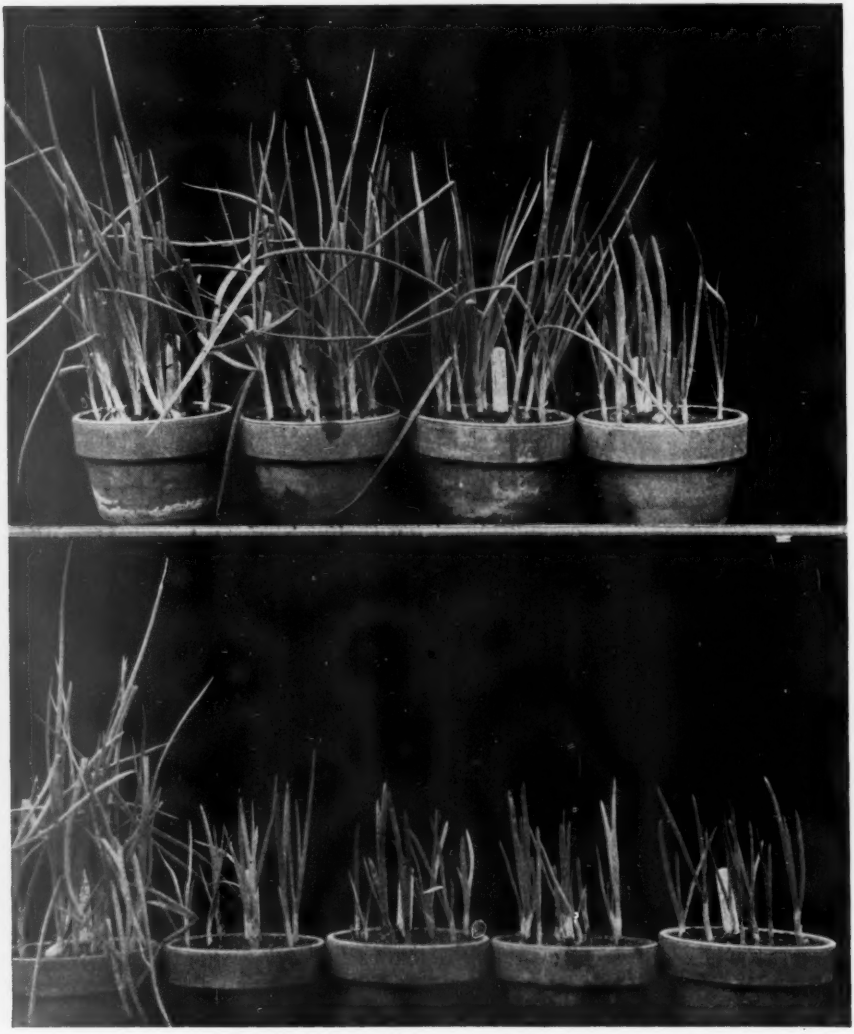
Internal effects

The trends in growth and mortality described above were found to be generally substantiated by a more detailed analysis of the cytological responses of the root-tip cells. In the present experiments, particularly at the higher dosage levels, the observed effects of both types of irradiation followed a pattern that was chronologically determined. In general, this sequence involved four stages: a general, but temporary, inhibition of mitosis; abnormal nuclear division necessitated by structural chromosomal aberrations; abnormal cell division directly following aberrant nuclear division; and, lastly, degeneration of nuclei and cells which had been severely damaged either by the original treatment or by disorientation in normal cell processes during the preceding stages.

Inhibition of mitosis.—In contrast to the direct action of radiation in producing structural changes in chromosomes (to be discussed later), the indirect action of radiation characteristically produces an inhibition of division, as well as an alteration of the surface properties of the chromosomes so that stickiness and clumping occur with subsequent failure of further division. Changes of the latter type do not appear to be a result of localized damage to the chromosomes caused by the passage of ionizing particles through given points but rather seem to

be the result of general changes in properties of the entire chromosomal surface and of physicochemical equilibria in the cell as a whole (14).

Temporary inhibition of division was found in the 48-hour collection of root tips from bulbs exposed for 16 minutes to slow neutrons. At the 96-hour period, however, a few division figures were evident. Similar trends were found for both 4- and 8-minute treatments with slow neutrons and with the 10,000-r series.



FIGS. 1, 2.—Plants from irradiated onion sets 3 weeks after treatment. Fig. 1 (*above*), X-irradiated: *left to right*, control, 100 r, 1000 r, 10,000 r. Fig. 2 (*below*), slow-neutron-treated: *left to right*, control, 2, 4, 8, and 16 minutes.

The data on inhibition of mitosis for 48- and 96-hour collections are presented in table 1. It is apparent that the 10,000-r treatment was markedly more severe than either the 100-r or the 1000-r treatments, for in the former the average rate of division was but one-tenth that of the control, while in the latter instances the rates of division were not diminished for the 96-hour period. The 2-minute exposure to slow neutrons did not produce results significantly different from those of the control with respect to rate of division, but exposures of 4 minutes or more caused marked depression of mitotic activity. The degree of inhibition following the 16-minute exposure approximated that resulting from the 10,000-r treatment.

The average number of cells counted for median root-tip sections of the meristematic region showed direct correlation with rates of mitotic activity. At higher dosage levels, particularly in materials treated with slow neutrons, the lower the rate of division, the fewer the numbers of cells scored.

In the case of the material treated with slow neutrons, in addition to scoring energic and dividing nuclei, a breakdown of the stages of mitosis was made. It is apparent from these data (table 1) that, once the cells at any given treatment level could overcome the inhibition, relatively constant ratios of cells in the various stages of mitosis were maintained. For the highest dosages in both series at the earliest collection periods, the greatest number of cells in division were in prophase. This was consistent with the observation of marked inhibition of mitosis in these treatments and with the fact that the prophase extended over a longer interval of time than did other stages and hence tended to be more numerous at any given time. The increase by a factor of

TABLE 1

INHIBITION OF MITOSIS INDUCED IN ONION ROOT TIPS BY IRRADIATION

Treatment	Total cells	Mean no. cells/root tip	Av. % divid.	% normal anaphases	% prophase	% metaphase	% anaphase	% telophase
Control	35414	2083.2	11.5	100	66.1	13.8	9.9	9.7
	X-irradiation*							
100 r	30122	1585.6	11.6	76	.....	.....	.....	.....
1000 r	27540	1449.5	11.3	59	.....	.....	.....	.....
10,000 r	17576	925.1	1.1	0	.....	.....	.....	.....
	Slow neutrons†							
2 min.	15604	1040.3	10.8	15	61.6	12.0	7.6	18.8
4 min.	18970	903.3	4.3	4	66.9	8.7	6.3	17.2
8 min.	14984	832.4	4.4	4	74.2	8.5	5.0	12.4
16 min.	10251	569.5	1.6	0	67.6	7.0	8.6	20.0

* All dosages administered at constant intensity of 25 r/minute.

† All dosages administered at average intensity of 5.8×10^{10} slow neutrons/sq cm/sec.

two in the percentage of cells in telophase of the material treated with slow neutrons over control values might be explained by the extension of the duration of this stage following the occurrence of bridges and subsequent failure to organize daughter nuclei (fig. 32).

Indications, which need further investigation, were that the cell-enlargement phase of growth might also be affected by treatment with slow neutrons. Measurement of median sections of meristematic zones (comparable areas) of control and treated root tips showed that the same relative areas were maintained with fewer cells in treated plants than in controls. This trend was found also within the series treated with slow neutrons. It seemed that the cells at the higher levels of dosage were enlarged in comparison with control materials or with those treated with lower dosages of slow neutrons.

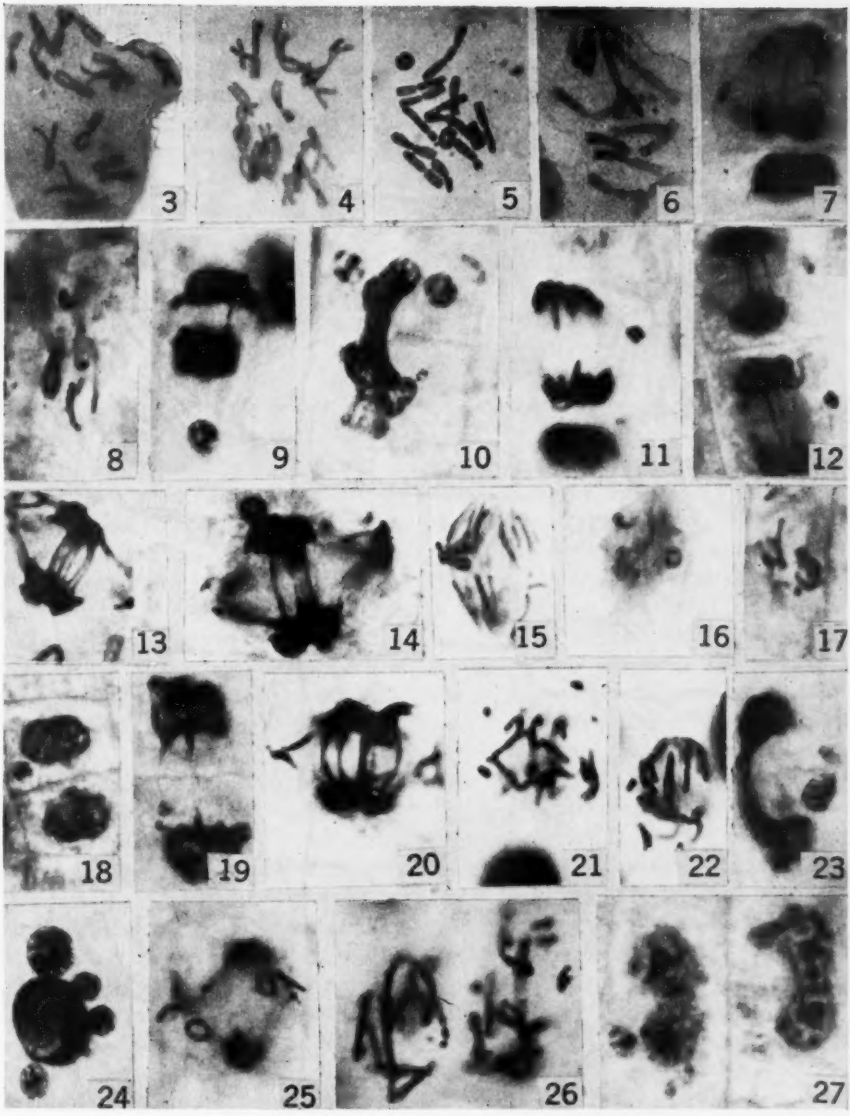
TYPES OF CHROMOSOMAL ABERRATIONS.—The action of radiation on living tissues has been described as being both direct and indirect (**11, 14**). Structural rearrangements of chromosomes result from the rejoining in various ways of breakage ends present in a nucleus in which chromosome breaks have occurred. This action of radiation appears to be direct, in the sense that the break is believed to be caused by ionizing particles passing through or in the immediate vicinity of the chromosome at the point where the break occurs.

The types of chromosomal aberrations found in both kinds of treated material were similar to those described by SAX (**22**: fig. 1) and LEA (**14**: figs. 30, 31). They may be classed under two general categories: chromosome aberrations and chromatid aberrations. Structural rearrangements resulting from breaks during the resting stage, or in early prophase before time of effective splitting of chromosomes, are termed "chromosome aberrations." Chromatid aberrations, on the other hand, are those resulting from breaks during the prophase after effective splitting of chromosomes into sister chromatids.

Since chromosome aberrations usually result when chromosomes are irradiated in the resting stage, only breaks of this type have been included. SAX (**24**) found no change in aberration frequency after a 3-week period of storage following irradiation treatment of onion bulbs. It has been reported (**19**) that spontaneous aberrations do not occur in the first division of root tips of sprouting onion bulbs —an observation confirmed by the absence of aberrations in the writer's control material. Hence, it is reasonable to assume that the structural changes here reported have been induced solely by the various treatments employed.

Chromosomal aberrations resulting from a single break include simple deletions and are detected by the presence of paired fragments at anaphase (fig. 11) or by a single bridge and accompanying fragment (fig. 7). Chromosomal aberrations resulting from two breaks are indicated by the presence of dicentric chromosomes at metaphase (figs. 5, 6), dicentric bridges at anaphase (figs. 12, 13, 14, 20), or ring chromosomes with their accompanying fragments (figs. 15, 16, 17, 22, 25). Translocations and inversions are rarely detected in *Allium* chromosomes.

The two-break chromosome aberration illustrated by figure 21, in which both centromeres of the dicentric chromosome were moving to the same pole, was a case infrequently observed, since the occurrence of this behavior is not readily detected in *Allium* chromosomes. Chromosomal aberrations resulting from

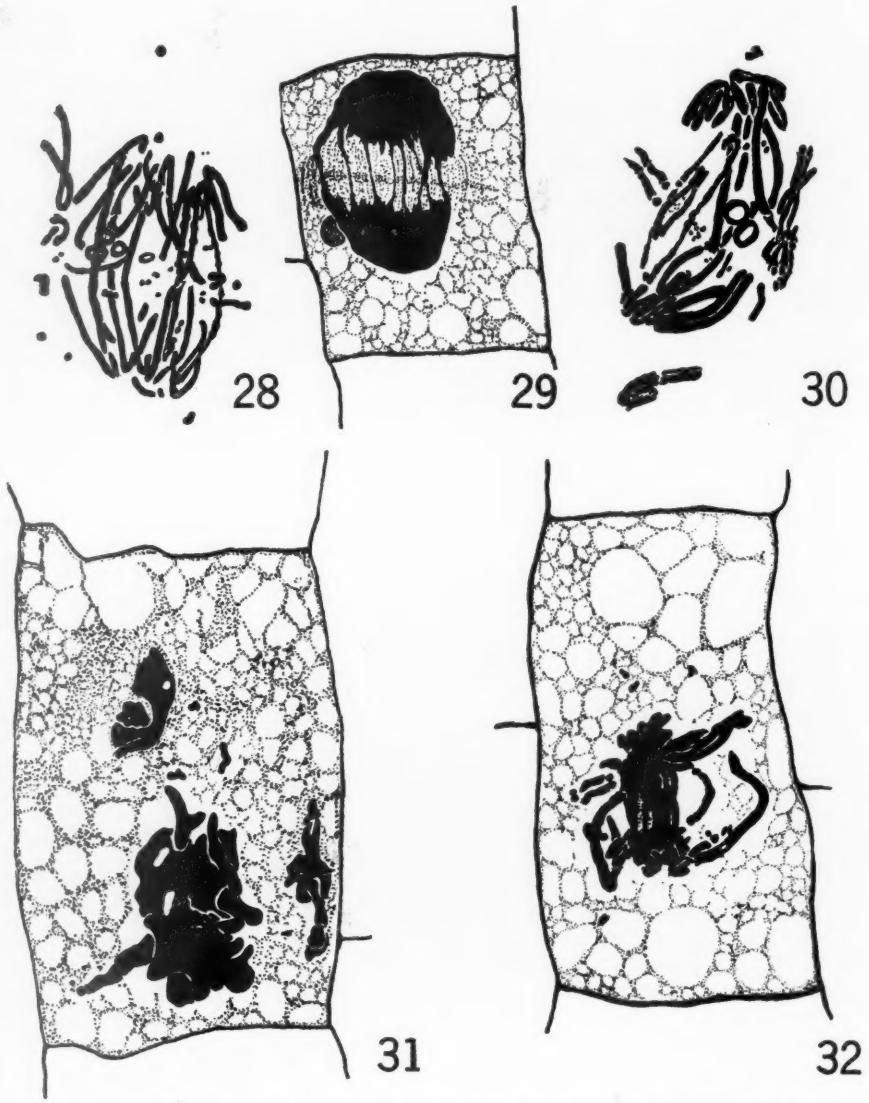


FIGS. 3–27.—Aberrancies in nuclear and cell division in onion root tips following irradiation of bulbs with X-rays (figs. 4–10) or slow neutrons (figs. 11–27). Figs. 3–5, ×600, oil; figs. 6–27, ×950, oil. See text for additional explanation.

multi-breaks are indicated by the occurrence of several polycentric chromosomes (figs. 8, 26).

Extreme examples of the number of types of chromosomal aberrations which may occur in a single cell are indicated in figures 28, 29, and 30, in which the complexity of the mixture interfered with accurate analysis.

Dicentric chromosomes separated



FIGS. 28–32.—Aberrancies in nuclear and cell division in 96-hour collection of onion root tips from bulbs irradiated with X-rays (figs. 30–32) or slow neutrons (figs. 28, 29). Drawn at table level with camera lucida; ×950, oil.

freely in only a few cases, in about two-thirds of the instances formed individual bridges, and in the remainder of the cases formed criss-crossed bridges or became interlocked at anaphase. The centric-ring chromosomes may form a single large ring (fig. 17), may separate freely (fig. 25), or may become interlocked (fig. 22), depending upon the amount of twist of the chromosome which has occurred between breakage and rejoining of the breakage ends. Acentric rings (figs. 5, 15, 16) and the acentric fragments associated with these or other types of aberrations lie free in the cytoplasm and are usually not included in the daughter nuclei, but may form micronuclei (figs. 9, 10, 18, 19, 23, 24, 27).

The ratio of dicentric to ring chromosomes was about 16:1. A broken end of a chromosome arm can fuse with a broken end in the other arm of the same chromosome to form a ring, or it can fuse with a broken end of any one of the thirty arms of the other fifteen chromosomes (for *Allium cepa*, $2n = 16$, fig. 3) to form a dicentric union. Thus, random reunion of broken ends should produce a ratio of dicentric to ring chromosomes of about 30:1. The discrepancy between the observed ratio and the expected ratio (about one-half) may indicate that spatial limitations (**22**), as well as movements both laterally and longitudinally with relation to polarized arms of the chromosomes, may condition or determine the observed occurrence of two-hit aberrations.

As a consequence of the direct action of radiation on chromosomes, in many cases the anaphase bridges were so numerous (figs. 13, 14, 29) that they could not be severed by the extension of the cell plate. In some instances of abnormal cell division following abnormal nuclear division (figs. 10, 23, 27) the cell plate extended across only half or less of the cell width. The appearance of these nuclei suggests amitosis, but, when the sequence of events leading to their formation is considered, it is evident that they are best designated as pseudo-amitotic nuclei (**10, 20**).

In materials given both types of treatment degeneration of nuclei and cells (figs. 31, 32) was of common occurrence. Karyopycnosis (fig. 31) and karyocholosis (fig. 27) were evidences of necrosis particularly marked at the higher levels of dosage of X-rays and slow neutrons.

ABERRATION FREQUENCY.—In table 2 data are presented on the frequency of chromosomal aberrations induced by irradiation. In both series the aberration frequencies of dicentrics, rings, and fragments showed a direct relationship with magnitude of dose. In the slow-neutron series all responses were more marked than were those for materials given 100 r or 1000 r of X-irradiation. It was only in the 10,000-r series that the aberration frequency approximated that found for the 16-minute slow-neutron dosage, indicating again that these two treatments were relatively equivalent in the degree of severity of damage induced.

When the number of aberrations per cell is plotted against the log of the dosage expressed as neutrons/sq cm (fig. 33), the magnitude of the increase of aberration frequency with increase in dosage is greater when the fragments are included. This is of significance in describing the total phenomenon, for the major difference observed among the various treatments with regard to structural changes induced was the greater occurrence of fragments at the higher dosages. It should be noted that these fragments were not those associated with the production of interchanges such as dicentrics or rings but constituted a class in themselves of small, isodiametric, or rod-shaped fragments and dots or interstitial

deletions. As indicated by the greater discrepancy between the two curves at correspondingly higher dosages, the preponderance of fragments indicated a greater total response than did the occurrence of dicentrics and rings only, at the same dose. This "peppering" effect, i.e., the production of many small fragments, was characteristic of the effect of when fragments were included than when only dicentrics and rings were plotted.

MITOTIC ACTIVITY AND EXPRESSED ABERRATION FREQUENCY.—The relationship between inhibition of mitosis and expressed aberration frequency is indicated by the data of table 3. The percentage of aberrant cells (which included structural rearrangements in aberrant

TABLE 2

FREQUENCY OF CHROMOSOMAL ABERRATIONS INDUCED IN ONION ROOT TIPS BY IRRADIATION

Treatment	Cells scored	Dicentrics	Rings	Fragments	Dicentric+ring aberr /cell	Dicentric+ring+fragment aberr./cell
Control....	4800	0	0	0	0	0
	X-irradiation*					
100 r...	1200	119	8	362	0.11±.01†	0.41±.02†
1000 r...	1200	306	15	568	0.27±.02	0.74±.03
10,000 r...	400	614	61	1592	1.69±.07	5.67±.12
	Slow neutrons‡					
2 min.....	2970	2384	192	2045	0.87±.02†	1.57±.02†
4 min.....	1622	1945	75	1760	1.25±.02	2.33±.04
8 min.....	1133	1673	70	2199	1.54±.04	3.48±.06
16 min.....	259	414	42	1198	1.76±.08	6.39±.16

* All dosages administered at constant intensity of 25 r/minute.

† Calculated from: sampling error = $\sqrt{n}/T$, where n = total number of aberrations and T = total number of cells scored.

‡ All dosages administered at average intensity of 5.8×10^{10} slow neurons/sq cm/sec.

the most severe treatments on the cells in both series.

If the number of aberrations per cell per neutron is plotted against exposure period (fig. 34), it is seen that total dosage (exposure time) and effect induced per neutron were inversely related. These curves would seem to indicate the operation of a saturation factor which effectively limited the number of observed aberrations. Again, as noted previously, the magnitude of effect was more marked anaphases, pseudo-amitotic nuclei, and cells possessing one or more micronuclei) was positively correlated with dose in both treatments. The apparently small number of total aberrant cells at the highest dosages for both treatments may be correlated with correspondingly lower rates of division at these dosages. This apparent saturation effect may be attributed largely to the fact that marked inhibition of mitosis made it impossible to score all cells affected. Physiologically

damaged cells appear energic and hence cannot be scored for structural chromosomal aberrancies. Over 20% of the cells of root tips treated with slow neutrons contained indication of aberrancies, whereas only 3% of X-irradiated cells showed abnormalities of any kind. It is possible that these relative incidences

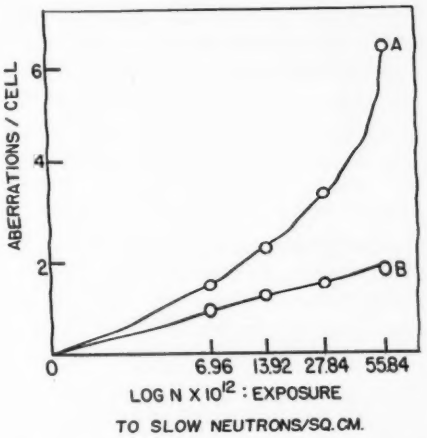


FIG. 33.—Aberration frequency as influenced by total dose of slow neutrons. *Curve A:* aberrations/cell (total). *Curve B:* aberrations/cell (dicentrics and centric rings only).

might be higher at later collection periods, for if materials given 10,000 r or 16 minutes of slow-neutron exposure recovered to a normal rate of division, the expressed abnormalities might be considerably more numerous than they were for the 96-hour collection.

These trends of the influence of inhibition of mitosis upon expression of aberration frequency are shown by the breakdown of percentage of cells in division, percentage of abnormal cells, and the dicentric plus ring aberration per cell at each collection period to demonstrate the importance of the time factor in studies of this type. The sequence of the indirect or physiological action of radiation preceding and, indeed, controlling the expression of the direct action in inducing structural changes is readily apparent.

Data from table 3 indicate that the percentage of abnormal cells showed a direct relationship to dosage with respect to time. Aberration frequency based on dicentric and ring chromosomes, on the other hand, showed positive correlation at higher dosage levels but an inverse relationship with respect to time.

LETHALITY OF TREATMENTS.—As noted earlier, all plants treated with slow neutrons or with 10,000 r showed marked retardation in growth and eventually died.

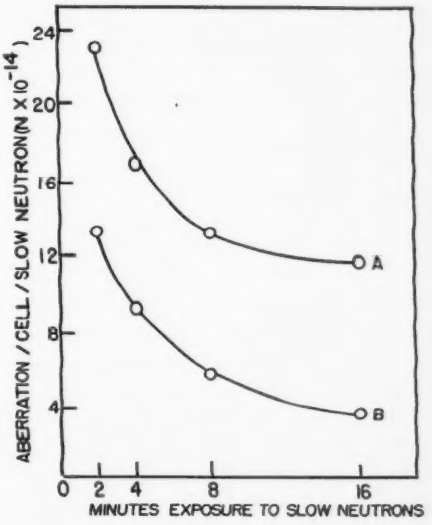


FIG. 34.—Aberration frequency per neutron at different exposure periods. *Curve A:* aberrations/cell (total). *Curve B:* aberrations/cell (dicentrics and centric rings only).

This suggests a direct correlation between the degree of abnormality produced in the root tips by the several treatments and failure to maintain normal growth and development in the plant as a whole. With regard to the high degree of correlation between the frequency of chromosomal

aberrations in root tips and the extent of shoot growth, it would seem that a level of one and a half total aberrations, i.e., rings and dicentrics, per cell is lethal for onion, whether the aberrancy be induced by X-rays or by slow neutrons.

The mean lethal, or inactivation, dose, which corresponds to an average of one hit per target, has been described by LEA (14) as the 37% dose. Analyses of the percentages of normal and abnormal anaphases at the various treatments employed (table 1) indicate that for X-rays the 37% dose may be obtained with about 3000 r. For the material treated with slow neutrons, since so few normal anaphases (15.2%) were found at the lowest (2-minute) exposure, the 37% dose can only be approximated to be about 1.9×10^{12} slow neutrons/sq cm. With the average neutron flux obtained at the pile power used in the present experiments this would correspond to an exposure of about 30 seconds.

DOSAGE-CURVE EXPONENTS.—To determine the relationships between dosage level and frequency of induced chromosomal aberrations in the present experiments the value of n in $(D/K)^n$ (where D is dosage; K, a constant; and n, the

TABLE 3

RELATIONSHIP OF INHIBITION OF MITOSIS AND ABERRATION FREQUENCY INDUCED BY IRRADIATION

Treatment	Collection period (hr.)	% dividing cells	% total aberr. (all types)	Dicentric + ring aberr./cell
Control	48	12.8	0	0
	96	10.6 Av. 11.5	0	0
		X-irradiation*		
100 r	48	10.4	0.78	0.12
	96	12.9 Av. 11.6	0.72	0.10
1000 r	48	11.3	2.69	0.33
	96	11.2 Av. 11.3	2.54	0.20
10,000 r	48	0.1	0	0
	96	2.1 Av. 1.1	1.31	1.69
		Slow neutrons†		
2 min	48	8.9	4.13	1.67
	96	12.8 Av. 10.8	17.76 Av. 13.02	0.81
4 min	48	1.9	0.70	0.96
	96	6.7 Av. 4.3	15.20 Av. 12.10	1.28
8 min	48	0.1	0	0
	96	8.7 Av. 4.4	20.57 Av. 20.57	1.38
16 min	48	0	0	0
	96	3.1 Av. 1.6	14.30 Av. 14.30	1.76

* All dosages administered at constant intensity of 25 r/minute.

† All dosages administered at average intensity of 5.8×10^{10} slow neutrons/sq cm/sec.

dosage-curve exponent) was obtained from the formula used by NEWCOMBE (**18**): (log effect 2/log effect 1)/(log dose 2/log dose 1). The chromosomal aberrations which involve two chromosomes or two loci of the same chromosome were found to increase in frequency approximately as the 0.6 power of the dose for materials treated with either X-rays or slow neutrons. The discrepancy between the values reported here and those reported elsewhere (**3, 26**) will be discussed in the following section.

Discussion

FROLIK and MORRIS (**5**) have observed that the percentage of F_1 plants with abnormal pollen was positively correlated with length of time of irradiation of maize tassels with slow neutrons. All plants in the progeny after a 16-minute exposure showed abnormal pollen. They suggested that this response might well be related to chromosomal aberrations induced by the treatment. Although percentages of abnormal F_1 plants were greater with added length of treatment, the degree of abnormality in affected plants did not vary for different treatment levels.

Results of the present experiments, both with respect to gross effects of treatment with slow neutrons upon total growth and to the cytological abnormalities described, generally corroborate these findings. Higher percentages of abnormal anaphases, greater aberration frequency, and more pronounced inhibition of division with longer exposure periods were found to show a high degree of correlation with the general retardation of shoot growth. Lethal effects of the highest dosage levels were manifest in the death of all exposed plants. Apparently maize pollen, which was able to give rise to an F_1 generation even though stands were drastically reduced, is somewhat less sensitive to a 16-minute exposure to 7×10^{10} slow neutrons/sq cm/sec than are onion sets exposed to 5.8×10^{10} neutrons/sq cm/sec for a similar period of time.

Observations on onion root tips following treatment of bulbs directly corroborate the marked inhibition of division described by GRAY and READ (**8**) for irradiated root tips of broad bean. In the present experiments no evidence for division of cells could be found to account for the extent of elongation of roots from bulbs at the higher levels of treatment. Inhibition of mitosis, particularly after exposure of 16 minutes to slow neutrons, in conjunction with the suggested hypertrophy of cells at this dosage, indicated marked retardation of the nuclear division phase of growth with no apparent effect on the cell enlargement phase of growth. The possible divorce of nuclear division from cytoplasmic elaboration should prove this interesting material for future cytochemical studies.

The differential response of nuclear division and cell enlargement is correlated with the greater sensitivity of the nucleus than of the cytoplasm to irradiation treatment (**24**). Studies reported by MCDONALD (**16**) suggest that nucleic acid (large molecular weight) may be broken into pentose nucleotides of smaller molecular weights following irradiation with fast neutrons. Lighter molecules might diffuse in the cell environment when the substance of larger molecular weight could not. Further, inhibition by these nucletides of the enzyme ribonuclease might interfere with the synthesis of the nucleic acid necessary to nuclear and cell division. MITCHELL (**17**) has assumed that derangements in nucleic acid structure are responsible for the temporary suspension of cell division following irradiation.

His theory that ribosenucleic acid can no longer be transformed into desoxyribosenucleic acid has been substantiated by his demonstration of an accumulation of ribosenucleic acid following X-irradiation. It has been suggested by LAWRENCE and JONES (13) that interference with nuclear metabolism may be the basic reason for inhibition of mitosis. Their findings confirm those of MITCHELL with respect to disruption of the nucleic acid cycle.

The marked pycnosis of some cells at 10,000 r and at the 16-minute exposure to slow neutrons is particularly interesting because it appeared only in the 96-hour collection and not earlier at these treatment levels. From the observation of HOHL (10) chromosomal pycnosis is apparently a nonspecific process, since it may result from ionization induced by irradiation, or by means of chemical reactions, or through dehydration procedures. The pycnosis he described was attributed to condensation of thymonucleic acid. This can best be explained by the hypothesis of LEA (14) in which it is assumed that the polymerized form of nucleic acid can be transformed relatively easily, by means of radiation or other agents, into the nonpolymerized, or so-called "sticky" or "clumped," form. Hence, some of the abnormal nuclear divisions found after the 16-minute exposures to slow neutrons might be ascribed to the physiological effect of radiation, rather than to the direct action upon chromosome structure. The accuracy of the aberration frequencies reported for this treatment may then be disputed on the basis that a mixture of physiological and structural changes were included. The anaphase figures analyzed, however, were restricted to those in which definitive structural changes could be observed; cells in which chromosomes showed stickiness or clumping were not included.

The possibility does exist, however, that the observed response of pycnotic cells (fig. 32) did not result from the general physiological action of the radiation treatment but could be due to extreme numbers of structural changes, with the production of so many interconnected bridges or interlocked rings that division could not proceed normally. The presence of nuclear material at either pole in such a cell would seem to suggest that some chromosomes, or centric remnants of chromosomes, were able to proceed through the various stages, while the majority of the chromosomes, severely disoriented by the complexity of structural changes induced, remained static or appeared pycnotic.

WHITE (28) has stated that many nuclei when injured or dying become pycnotic; that is to say, their chromosomes become fused into a single large mass which stains intensely with dyes such as haematoxylin. Further, pycnosis is probably to be interpreted as a highly modified, nonfunctional mitosis, since in some cases pycnotic nuclei may begin to divide.

There are thus three possible explanations for the pycnotic appearances observed in nuclei. The data here suggest that any one hypothesis may be applicable in some instances and not in others. For the total observed responses leading to the formation of pycnotic nuclei, all three explanations must be considered.

The occurrence of pycnotic and degenerating nuclei or micronuclei in the 96-hour collection (figs. 7, 27, 29, 30) for several of the treatments constituted evidence of necrotic changes in these cells. Such changes may indicate differential survival of cells which have been subject either to severe damage by the original

treatment or to deletions and other genomic unbalance as the result of structural changes and subsequent abnormal division (**19, 23**). PFEIFFER (**20**) has described abnormal nuclear division in which mitosis does not proceed to telophase. These cells die, showing granular or vacuolar necrosis.

The nucleus with five micronuclei (one is below the level of focus) shown in figure 24 would seem to be of the "budding" type described by DOXEY and RHOADES (**4**) for onion root tips treated with methoxone. When, however, the apparent sequence of events leading to the formation of such an anomaly is considered, it is evident that this may not be amitosis but pseudo-amitosis. PFEIFFER (**20**) has described atypical mitosis resulting from treatment by irradiation in which fragments resulting from dicentric or ring formation lie free in the cytoplasm. He considered that these may be lost into the cytoplasm or may form a micronucleus, which in most cases is soon resorbed. Thus the budding nuclei may be interpreted, not as expulsion of nuclear material, but as resorption of that material which has remained in the cytoplasm following abnormal nuclear division.

SAX and BRUMFIELD (**26**) have reported on the differential sensitivity of cells to X-rays. They found that root-tip chromosomes of *Tradescantia* are less sensitive than are the microspore chromosomes of similar plants by a factor of about 1:4 at 300 r, assuming a linear dosage curve for the root-tip chromosomes. A comparison of their reports for chromosomes of onion root tips and those of *Tradescantia* microspores shows a lower sensitivity of onion chromosomes by a factor of about 1:10 at the 300-r level. As partial explanation for the relation between X-ray dosage and two-hit chromosomal aberrations and the inherent sensitivity of various plants or plant parts, these authors stated (**24, 26**) that even moderate doses of X-rays inhibit nuclear division in root tips for 24 hours or longer. The retardation of nuclear activity should inhibit chromosomal movement and reduce the frequency of two-hit aberrations because broken ends would be more likely to undergo restitution, instead of getting out of normal alignment and fusing to produce rings and dicentrics. This physiological action of X-rays would result in essentially the same effect as that induced by the use of colchicine prior to irradiation (**15**). The relationship between inhibition of mitosis and aberration frequency as described earlier for the present experiments indicates the operation of such a saturation factor, for the degree of retardation of nuclear activity by irradiation appeared to be directly related to dosage. Such a relation in root-tip cells would tend to result in a relatively lower observed aberration frequency at higher doses than might be expected. If the number of breaks actually present could only be minimally realized, as an inverse function of inhibition of mitosis at higher dosages, lower values than potentially expected would then subject dosage-curve exponents to a certain degree of experimental error.

The expected frequency of dicentric to ring chromosomes in onion is 30:1, if the broken ends of chromosomes were joined at random. SAX (**23**) has reported an observed ratio of 9:1. This value is about one-third of that expected. In the present experiments the observed ratio of 16:1 is a little over half the expected frequency. SAX has attributed the discrepancy between observed and expected ratios to spatial limitations in unions of broken ends of chromosomes. It would seem, however, that part of the discrepancy

might well be attributed to his 24-hour treatment of root tips with colchicine prior to fixation (15). If, as SAX (24) stated, it seems probable that breaks remain open for many hours, or perhaps indefinitely in the completely dormant resting nucleus, and that fusions occur only with the initiation of nuclear activity, a prolonged prefixation treatment with colchicine could effectively immobilize chromosomes and so favor intrachanges (ring formation) rather than interchanges (dicentric formation).

Frequency of two-break chromosomal aberrations in *Tradescantia* microspores are a function of the square of X-ray dosage when the radiations are given at high intensities or when the time of exposure is constant (6, 14, 24). These results are to be expected if aberrations which involve two different chromosomes or two loci of the same chromosome are the result of two independent breaks produced by two separate hits (14). This interpretation has been supported by comparison of aberration frequencies induced by X-rays and fast neutrons (6, 14, 27).

The dosage curves described by SAX (23) for X-irradiated onion seedling root tips indicate that aberration frequency increased approximately as the 1.5 power of the dosage. In the present experiments the dosage-curve exponent was found to approximate the 0.6 power for both the higher dosages of X-rays and all levels of the slow-neutron treatment. This apparent discrepancy may be variously attributed to the colchicine treatment employed by SAX, to pre-irradiation history of the bulbs, and to varietal differences in the material used. SAX did not report the variety or varieties of bulbs used in his investigations.

NICHOLS (19) has suggested that inherent differences in susceptibility to chromosomal alterations exist in the several varieties of onion. Such inherent differences in the capacity of cells and tissues responding to injuries after radiation damage may reconcile the discrepancies noted above. MCDONALD (16) has stated that organisms irradiated with a lethal dose usually survive for a period and then die. Tissues are capable of repairing injury produced when the dose is small, and so it may be counteracted. When, however, the dose is sufficiently large, the repair processes produce a negligible effect upon the total amount of injury, so that at higher intensities the biological effect of radiation is independent of the intensity.

General discrepancies, therefore, between the reports of SAX (23, 26) and others for irradiation of dormant onion bulbs and the data of the present experiments may be attributed in large part to the extremely high dosages employed. The lethality of the treatments may account for the low dosage-curve exponents obtained, for with the greater percentage of cells injured so severely that marked inhibition of division occurred, the number of two-hit chromosomal breaks could be approximated only minimally.

From these experiments, then, it would appear that, although one can ascertain qualitative relationships between excessive treatment and the induced radiobiological effects, aberration frequency alone does not yield a satisfactory or valid quantitative index of the true picture of dosage effect.

Summary

1. Bulbs of *Allium cepa*, variety Ebenezer, were exposed to X-ray dosages of 100 r, 1000 r, or 10,000 r, or to slow neutrons produced in the Argonne heavy-water pile. The neutron-treated material was exposed to an average neutron flux

of 5.8×10^{10} slow neutrons/sq cm/sec for periods of 2, 4, 8, or 16 minutes.

2. Plants from treated bulbs in both series were markedly retarded in growth with respect to control plants. All plants from bulbs treated with slow neutrons died as did also those treated with the highest dosage of X-rays.

3. Bulbs from control and treated series were sprouted in soil. Cytological analysis of root tips collected at 48-, 52-, and 96-hour intervals showed that the effects of both types of treatment followed a pattern that was chronologically determined: a general, but temporary, inhibition of mitosis; abnormal nuclear division necessitated by structural chromosome aberrations; abnormal cell division directly following aberrant nuclear division; and, lastly, degeneration of nuclei and cells which had been severely damaged either by the original treatment or by disorientation in normal cell processes during the preceding stages.

4. Chromosomal aberrations of all types—dicentrics, centric and acentric rings, acentric fragments, and dot deletions—were observed in root-tip cells from both treatments. Dosage had no apparent effect on type of aberrancy induced. Pycnosis of many nuclei at the higher dosages was of common occurrence.

5. Aberration frequency induced by 2-, 4-, or 8-minute exposures to slow neutrons was higher in all cases than that produced by either 100 r or 1000 r. Frequencies in the 10,000-r series approximated those found for the 16-minute dosage with slow neutrons.

6. The degree of fragmentation produced at the higher treatment levels by both X-rays and slow neutrons gave a more accurate picture of the shattering effect on chromosomes than did the extent of formation of dicentrics and rings.

7. The observation of pseudo-amitotic nuclei and daughter nuclei accompanied by micronuclei does not constitute evidence of induced amitosis as a result of treatment. These are stages in a time continuum of the general effects of radiation on chromosome breakage, abnormal nuclear division, and subsequent abnormal cell division.

8. The marked inhibition of mitosis found in the present study and the low dosage-curve exponents obtained for aberration frequency indicate that with regard to quantitative determination of the relationship between excessive treatment with slow neutrons and response of the organism, aberration frequency alone does not yield a wholly representative or valid index of the true picture of dosage effect.

The writer expresses her gratitude to Dr. N. J. SCULLY for obtaining and irradiating the material used and for providing survival data; to Drs. C. E. OLMSTED, J. R. RAPER, N. J. SCULLY, and R. W. RASCH for reading the manuscript and for suggestions offered; and to Dr. J. M. BEAL for his constant interest, helpful criticism, and invaluable advice throughout the course of this investigation.

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COMPARATIVE SEEDLING CHARACTERISTICS OF STANDARD CRESTED WHEATGRASS AND RUSSIAN WILD-RYE[1]

L. D. POTTER

Introduction

This study is concerned with the seedling stages of two grasses especially adapted to introduction and culture in the northern Great Plains area. Crested wheatgrass (*Agropyron cristatum* [L.] Gaertn.—hereafter referred to as *Acr*) is a typical, perennial bunchgrass without root-stocks; it is hardy, drought- and frost-resistant, and long lived. It was first introduced in America in 1897–1898 by N. E. HANSEN from samples received at the Valuiki Experiment Station, Samara Government on the Volga River, being native to the cold, dry plains of Russia and Siberia. Its history in North America has been reviewed (**1**), and there is an abundance of literature on the value of this grass in range and pasture seeding.

Russian wild-rye (*Elymus junceus* Fisch.—hereafter referred to as *Eju*) is a drought- and frost-resistant grass, widely variable in vegetative growth, but of a bunch type with erect culms and an abundance of basal leaves. One of its earliest introductions into this country was in 1927 from the Western Siberian Experiment Station, Omsk, U.S.S.R., as S.P.I. 75737. Its history has been reported by ROGLER (**11**). According to the distribution given by NEVSKI (**8**), this grass is native to dry, saline soils of steppes and steppe slopes with a climate of hot summers, cold winters, and low precipitation. It is found in some of the driest areas of Russia having about 4 inches of precipitation per year.

The native range of both grasses makes them suited to the northern Great Plains where they are best planted late in the fall, or as soon as possible in early spring. Crested wheatgrass (*Acr*) has been used extensively for reseeding thousands of acres of abandoned land, while the potentialities of Russian wild-rye (*Eju*) have only recently been recognized. Neither grass is so fruitful when self- as when cross-pollinated. Both grasses begin growth in very early spring and are suitable for grazing 3–4 weeks before the native range. *Acr* produces some seed the first season, while *Eju* does not yield grain until the second year and never produces seed so well as *Acr*. Although the forage production of *Eju* is usually not superior to that of *Acr*, the former begins growth earlier in the spring, continues longer in the summer and fall, is more drought-resistant, makes a more rapid regrowth after cutting, and is more palatable with a higher digestibility than is crested wheatgrass (**4, 5, 7, 9, 13, 14, 15**). Although *Acr* excels native grass in livestock production, *Eju* has been shown to excel both types in trials in North Dakota (**14**).

The purpose of this study was to compare some of the important properties of the seedling stages which fit these two grasses to the conditions of the northern Great Plains.

Material

All seed was furnished by Mr. GEORGE ROGLER of the Northern Great Plains

[1] Paper no. 3, Journal Series, from the North Dakota Institute for Regional Studies.

Field Station at Mandan, North Dakota, *Eju* designated by Introduction no. D-19 and *Acr* as Mandan no. 98–9. Most of the experiments were done with seed harvested in 1945, which was then 2 years old, although some experiments included 1947 seed which was only 1–2 months old. The *Eju* seed was run through a hammer-type mill at slow speed to remove the sharp awn and stiff hairs of the lemma and palea. Most of the experiments were conducted at or near the University of Minnesota.

tion. Where alternations of temperature were employed, the first temperature was used for 16 hours and the second for 8 hours. The moisture content, weight per 1000 seeds, and germination results are included in table 1. The 1947 *Eju* seed was of light weight, indicating poor development.

The 1945 seed of *Eju* was superior in germination percentage to *Acr* (table 1). The germination percentages of the 1947 *Eju* seed were low, both at 1 month after harvest (Minnesota) and at 11 months after harvest (North Dakota), indicating that the first low germination percentage did not result from a required period of seed dormancy but from something different in the character of the seed. The 1947 seed of both species was harvested from a new planting made in 1946 from the same stock from which the 1945 seed was harvested. The viability of the seed was affected either by the newness of the planting, by some climatic condition preventing proper filling of the grain (which was not evident to the writer from the climatic data), or by some change in the time or method of harvesting. The best germination conditions for both species were a constant temperature of 20° C. preceded by a

TABLE 1

PERCENTAGE MOISTURE, WEIGHT, AND RESULTS OF GERMINATION TESTS (PERCENTAGE) OF SEED OF *Elymus junceus* (EJU) AND *Agropyron cristatum* (ACR)

SEED	% MOISTURE	WT. (GM.) 1,000 SEEDS	20°–30° C.* 14 DAYS	CHILL 6° C., KNO_3† 20°–30° C.		CHILL 6° C., KNO_3† 20° C.		NO CHILL, H_2O† 20°–30° C.		NO CHILL, H_2O† 15°–30° C.	
				8 days	14 days	8 days	14 days	5 days	14 days	5 days	14 days
Eju—1945	3.29	2.910	97	77	95	20	96	83	88	61	97
Eju—1947	2.92	2.620	77	14	67	57	74	59	68	32	72
Acr—1945	2.85	2.757	91	31	71	13	84	65	83	.....	.....
Acr—1947	3.08	2.846	92	60	88	42	99	85	92	.....	.....

* Minnesota Seed Laboratory, August, 1947.
† North Dakota Seed Laboratory, June, 1948.

Experimentation

STANDARD GERMINATION

Standard germination procedures of seed-testing laboratories were used on two ages of seeds of both species at the State Seed Testing Laboratory at University Farm, St. Paul, Minnesota. The germination results are from tests made with alternations of 20° C. for 16 hours in artificial and natural light with 30° C. for 8 hours in darkness (table 1). Seeds from the same sample were also tested at the North Dakota State Seed Laboratory, Fargo, using three types of germination conditions (table 1). The chilling at 6° C. was for a period of 4 days, with seeds moistened with a 0.2% KNO_3 solu-

6° C. chill or an alternation of 20°–30° C. without a chill.

Germination trials of the two species by others[2] have indicated that there is generally a higher percentage of germination and longer viability with seeds of *Eju* than with *Acr*.

reviewed by EDWARDS (2) and FRANCK (3).

Apparently sound seeds of both species which were 2 years or 1 month old were incubated in duplicate lots of two hundred seeds each between moistened blotters in Petri dishes in temperature cham-

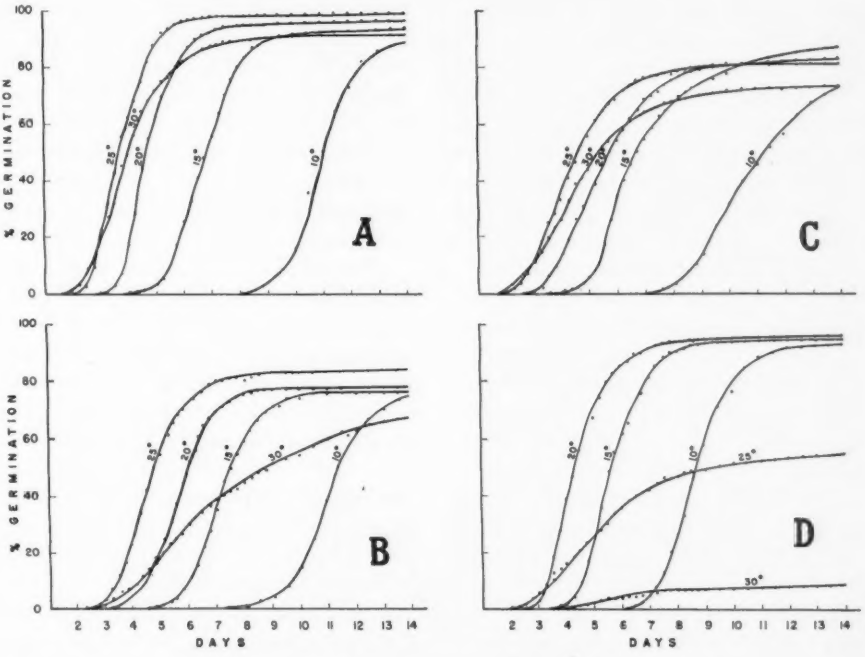


FIG. 1.—Time-temperature relationships of germinating seeds. Results from duplicate lots of two hundred seeds between moistened blotters in Petri dishes. Observations made every 6 hours until tenth day and every 12 hours thereafter. Seed samples used were: *A*, 1945 *Eju*; *B*, 1947 *Eju*; *C*, 1945 *Acr*; *D*, 1947 *Acr*.

TIME-TEMPERATURE GERMINATION RELATIONSHIPS

The Proceedings of the Association of Official Seed Analysts (1947) lists several procedures for testing *A. cristatum* but makes no mention of *E. junceus*. Much of the literature on the general subject of time and temperature relations has been

[2] Correspondence with Mr. GEORGE ROGLER, Agronomist, Northern Great Plains Field Station, Mandan, N.D.

bers at 5°, 10°, 15°, 20°, 25°, and 30° C., with a variation of ±0.1° C. Artificial light of low intensity was supplied throughout the experiment. Observations were made every 6 hours after germination began until 10 days, every 12 hours from 10 to 14 days, and again at 21 days. Germination was recorded when both radicle and plumule had emerged at least 2 mm.

For *Eju*, 1945 seed (fig. 1*A*), 25° C.

was the optimum temperature, resulting in a maximum daily germination rate and in maximum final percentage of germination. At 30° there was early germination of a few individuals but a slower daily increase in rate than at 25°, so that at 14 days the germination percentage was less than at all other temperatures except 10° and 5°. At 5° there was no germination of any seeds until the third week.

In general, the 1947 *Eju* seed (fig. 1*B*) did not germinate so early as the 1945 seed, the daily rate of germination was slower, and the final germination percentage was lower at all temperatures.

For the 1945 *Acr* seed (fig. 1*C*) the rate of germination during the first 24 hours was greater at 30° than at 25°, after which the rate at 25° excelled and was optimum for the first 10 days. The greatest final percentage of germination occurred at 15°, however, followed by those at 20°, 25°, 10°, and 30°. The data indicate germination requirements of cooler temperature for *Acr* than for *Eju*. These requirements for *Acr* are further exemplified by the 1947 tests (fig. 1*D*). The relative positions of the curves at 25°, 20°, and 15° in the 1945 *Acr* tests were replaced by curves for 20°, 15°, and 10°, respectively. The daily rate of germination as well as the final percentage of germination was greater for the 1947 seed than for the 1945 seed at these three temperatures, although the average final germination for all temperatures was 5% higher for the 1945 seed. The inhibitory effect of high temperatures (30°) on rate of germination and final percentage is apparent in all four lots of seeds.

The pregermination period—the time from beginning of incubation until initial germination—was not greatly different for the four samples (fig. 2). The 1945 lots of *Eju* and *Acr* were particularly similar. The length of the pregermination period was inversely related to temperature.

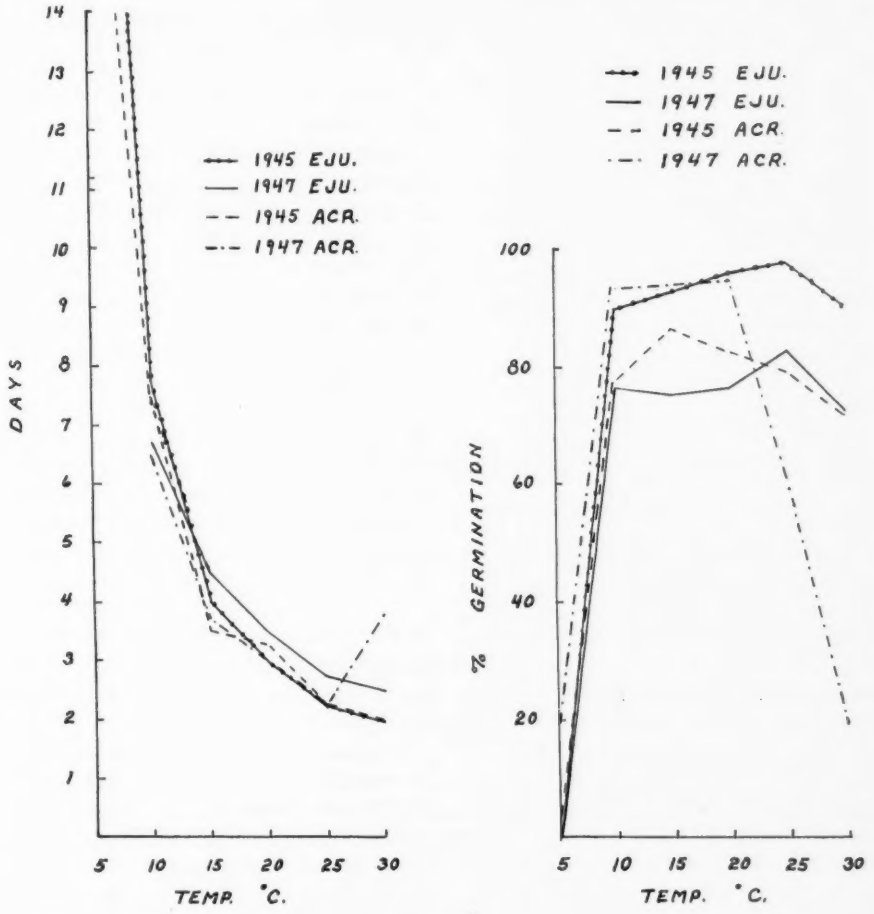
The final germination percentage (fig. 3) at 3 weeks is of interest because of the comparison of the two species at the conventional germination temperatures. The optimum temperature for germination of *Eju*, for both 1945 and 1947 seed, was 25°, with the results at 30° being approximately the same as those at 10°. The adaptability of *Acr* seed to germination at lower temperatures is again demonstrated with an optimum temperature of 15° for the 1945 *Acr* seed, while the optimum for the 1947 seed extended over the range 10°–20°.

The only samples to show any germination at 5° at the end of 3 weeks were of 1947 and 1945 *Acr*. The *Eju* seeds were then swollen but showed no sign of radicle or plumule emergence. At 4 weeks the *Eju* seeds had just started to germinate; by this time the seeds of all samples were covered with mold. By the end of 5 weeks the germination percentage of the 1945 *Eju* seed exceeded that of either age of the *Acr* seed. Seeds were not counted again until the end of 12 weeks; the 1945 *Eju* seeds then had the high percentage germination of 96%, followed in order by 1947 *Acr*, 81%; 1947 *Eju*, 74%; and 1945 *Acr*, 60%.

Rate and amount of imbibition of water

The rate at which water is absorbed into the seed affects the rate of germination. Quadruplicate lots of three hundred apparently sound seeds of the 1945 harvest of each species, which had been stored air-dry, were weighed on an analytical balance. The lots were then placed on uniformly moistened blotting papers in Petri dishes and kept at 20° C. At each successive weighing, at intervals of

1, 3, 5, 8, 12, 24, 36, and 48 hours, the seeds were brushed off the blotting paper with a tweezers into a dry glass weighing bottle and weighed to the nearest 0.0001 gm. They were then immediately replaced on the blotting papers and returned to the 20° C. temperature. The 20° temperature was used, since it was shown to be the second best temperature for both species in the time-temperature experiment as indicated by the percentage of final germination. A comparison employing the optimum for either species —15° for *Acr*, 25° for *Eju*—would have been unsatisfactory. The rates of imbibition, expressed either as gain in percentage of the original weight or as grams of water absorbed, were nearly alike for the



FIGS. 2, 3.—Fig. 2. (*left*), pregermination period. Time between beginning of incubation of seed and initial germination (emergence of both radicle and plumule to 2 mm.) of seeds used in time-temperature germination experiment. Fig. 3. (*right*), final germination percentages at 21 days. Data are from readings taken 1 week after results shown in fig. 1.

two species (fig. 4). The average original weight of three hundred *Eju* seeds was 26 mgm. greater than that of the *Acr* seed. The initial absorption and the increase of water absorption up to 36 hours were greater for *Acr* than for *Eju*. In the 12 hours following this period, however, the seeds of *Eju* gained rapidly in weight, reaching almost the same percentage as *Acr* at 48 hours. This is of special interest in view of the number of *Eju* seeds

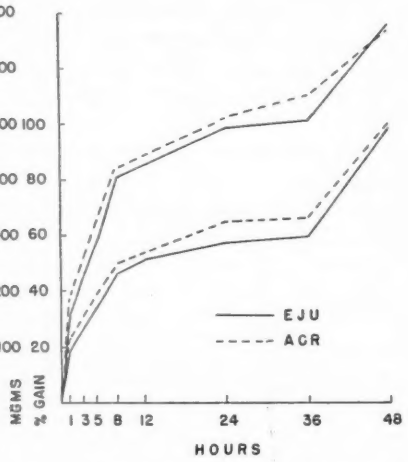


FIG. 4.—Rate of imbibition of water by seeds. Results represent weighings of lots of 300 sound seeds at 20° C. expressed as gain in percentage over original air-dry seed weight (*lower curves*) and as milligrams of water imbibed (*upper curves*).

which showed the emergence of the radicle at the end of the 48-hour period. For *Eju* seeds the average percentage of germination at that time for the four lots of three hundred seeds each was 60.8%; for *Acr*, 36.2%.

The water imbibed by *Eju* seeds, though smaller in amount, was thus more effective in bringing about germination as evidenced by radicle emergence. This earlier emergence may be an important factor in germination and seedling development in favor of *Eju*, for, when planted by broadcast or shallow drill, as is the practice with grass seed, there is danger of the surface soil drying out a short time after seeding, especially in the area where these two grasses are used. It therefore follows that the plants whose seeds can most quickly germinate and produce a root system have the advantage.

PERCENTAGE OF WATER NECESSARY FOR GERMINATION

It was next desired to learn the speed of germination of the two species at various moisture percentages. Triplicate samples of one hundred apparently sound seeds of the 1945 harvest for both species were spread out in the bottom of clean Petri dishes and covered with very fine, washed Ottawa quartz sand until the bottom of the dish was full. The sand had been made up to various moisture percentages—0.5, 1.6, 3.4, 8.2, and 22.0—from the oven-dry condition. A double thickness of wax paper and the cover of the dish were placed over the sand to prevent evaporation, and the dishes then inverted so that the seeds, now on the upper side, were exposed to the light. They were germinated at 20° C., ± 1° C., with alternating 12-hour light and dark periods, the light intensity at 15 foot-candles. In observations at 12-hour intervals germination was recorded when the radicle had emerged at least 2 mm.

The driest sand, 0.5% moisture on the dry-weight basis, was inadequate for germination of either species during the 17-day experimental period. The hygroscopic moisture percentage of Ottawa sand is about 0.1 %. Twice the hygroscopic moisture percentage is often given as the minimum at which seeds can germinate.

In all other samples (fig. 5) initial

germination of both species occurred in 2.5 days, except for *Acr* in the 1.6% sample. The period in which rapid germination took place was earlier and the rate of germination was greater with more moisture up to and including 8.2% moisture. In all these cases *Eju* was superior to *Acr* in using the available moisture to initiate germination.

Considering the time at which 75% germination was reached by the samples at 1.6%, 3.4%, and 8.2% moisture content, *Eju* germinated 33 to 51 hours earlier than *Acr*. This difference of 2 days becomes significant in relation to the period of 3–4½ days which it takes for *Eju* to reach 75% germination. Again, it is suggested that the more rapid germination of *Eju* should be of practical importance in seedling establishment in the plains area where lack of moisture near the soil surface is often a limiting factor.

The samples with 22.0% moisture appeared to have had water in excess and probably a deficiency of oxygen. The seedlings which did develop had yellowish plumules unlike the green ones of the other samples. The rates of germination were slow, but here again *Eju* exceeded *Acr* in final germination percentage.

Frost resistance of seedlings

Both species are of the cool-season type which commence growth very early in the spring and make much of their growth in a short period. They are often subjected to spring frosts in which the temperature may suddenly drop from that of growing conditions to well below freezing. It was thus desired to determine experimentally the effect of various freezing conditions at several growth stages of the seedlings. Included in the voluminous literature on frost resistance is a report by Rogler (12) in which *Acr* was found to be superior in frost resistance to several other cool-season grasses in the seedling stage.

Climatic data from Mandan, North Dakota, which was the source locality of the seeds used, show that the last frost in the spring may occur from 28 to 56 days after the date of emergence of *Eju*,

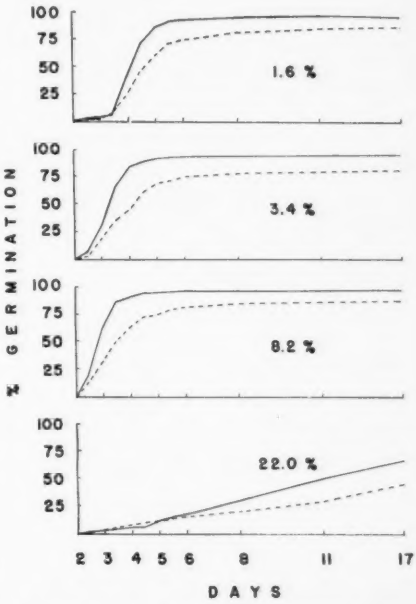


Fig. 5.—Effect of available substrate moisture on rate of germination; medium was fine Ottawa quartz sand. Alternating 12-hour periods of light and dark at 20° C. were used. Groups of curves represent results at various moisture percentages on dry-weight basis. No germination occurred at 0.5% moisture. Solid lines, 1945 *Eju* seed; broken lines, 1945 *Acr* seed.

which would be essentially the same for *Acr*. The minimum temperature during this last frost in the years 1936–1946 has been as low as 10° F. (—11° C.), almost equal to the freezing temperature used in this experiment. Often the last frost is preceded by several weeks of mild growing weather and will occur suddenly with little chance for hardening to take

place. Because of the earliness of spring seeding of these grasses, a late frost may occur any time up to 2 months after germination. Fall plantings are of two types: those made before the latter part of September which allow some autumnal growth and those made after that time in which the seeds lie dormant over winter but germinate early in the spring. Seedlings from the latter type of fall planting would be subjected to conditions similar to those used in this experiment, as would those from spring plantings.

Seedlings were grown in potting soil in flats in alternating rows of wheatgrass and wild-rye which were 1 inch apart with plants $1\frac{3}{8}$ inches apart in the rows—fifty plants of each species per flat. These were grown outdoors starting in mid-May and under good growing conditions. The flats were kept weeded to insure uniformity of competition. The plants were tested for frost resistance, at four ages after planting, in a low-temperature research laboratory of the Agronomy Department, University of Minnesota Farm Campus. Additional variables were length of precooling period, length of freeze, and treatment after freezing. After freezing, the plants were thawed at 38°–46° F. except in one treatment. During 12-hour periods the plants were illuminated by two 100-watt "daylight" fluorescent lights placed about 4 feet above them or subjected to alternate periods of darkness (table 2). Before each freeze treatment, counts were made of the percentage germination, number of living plants, growth stage (based on number of leaves), and height of each plant. In all cases *Acr* exceeded *Eju* in both total height of seedlings and in number of leaves per plant. Various observations were made on soil temperatures during freezing, the appearance of the frozen plants, the reaction to thawing and recovery, in addition to counts of the number surviving or recovering from the freezing treatment.

As the seedlings reached various ages —17, 33, 55, and 69 days—any one flat was subjected to one of the freezing treatments. Because of the extreme severity of treatments B and C with the first group of seedlings, two modifications (D, E) were substituted, giving a total of five different treatments.

TABLE 2

SUMMARY OF SEEDLING AGE AND METHOD OF TREATMENTS FOR TESTS OF SEEDLING FROST RESISTANCE

Treatment	Age of seedlings (days)	Precooling	Frost		Thaw		
			Hr.	Temp. (° F.)	Hr.	Temp. (° F.)	Light
A	17, 33, 55, 69	None	6	10°–14°	12	38°–46°	In light
					12	38°–46°	In dark
B	17, 55, 69	None	12	10°–14°	12	38°–46°	In light
					12	38°–46°	In dark
C	17	None	12	10°–14°	12	38°–46°	In light
			12*	10°–14°	12	38°–46°	In dark
D	33	12 hr. at 38°–44° F.	6	10°–14°	12	38°–46°	In light
E	33, 55, 69	None	6	10°–14°	†	†	†

* Repeated.

† None, return to outdoors.

The rapid changes in soil temperature which occurred in transferring flats of seedlings from outdoors to the freezing room are indicated in table 3, which is one of several series of temperature data obtained from thermometers buried at different depths in the soil. It should be emphasized that the experimental soil temperature conditions were more severe than would be encountered in the field, since there was considerable cooling from below as well as from the soil surface. All the soil in the flat became cooled and within 4 hours had reached the freezing point to a depth of 2 inches.

TABLE 3

SOIL TEMPERATURES DURING 6-HOUR FREEZE

Time (hr.)	Depth (inches)				Air temperature (soil surface)
	0.5	1.0	1.5	2.0	
0	18° C.	18° C.	18° C.	18° C.	−10° C.
0.5	10	11	13	14	−10
1	6	7.5	9	10	− 9.5
1.5	3.5	4	6	6.5	− 9.5
2	1.5	2	3.5	4	−10
2.5	1	1	2	2.5	−10.5
3	0.5	0.5	1	1.5	−10
3.5	0	0.5	0.5	0.75	−10.5
4	0	0	0	0	−10.5
4.5	0	0	0	0	−10.5
5	0	0	0	0	−10.5
5.5	− 0.5	− 0.5	0	0	−10.5
6	− 0.5	− 0.5	0	0	−10.5

The frost resistance—the ability of a plant to survive increasing intensity of frost—of *Eju* and *Acr* seedlings is summarized in figure 6. The bar graphs represent the percentages of seedlings which survived the freezing treatment, based on the number of living seedlings in each flat at the time of freezing. Readings were made 5–7 days after freezing, except that 69-day-old seedlings were not counted until after 16 days.

Most plants which did not survive turned dark green shortly after thawing out, became permanently wilted, and the leaves became curled as they dried. Of those that survived, the reactions were varied. Some plants were little harmed, and entire leaves became functional with normal green color and turgidity. In most survivors, however, much of the leaf area was killed; and after a few days new growth appeared from the basal meristems. In plants having a number of leaves, the older leaves were least apt to survive, while the basal portions of the upper leaves had the highest survival. In both species many plants produced new leaves within several days after freezing.

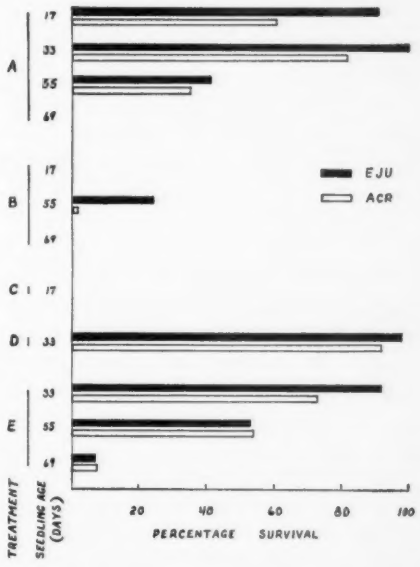


FIG. 6.—Survival of various aged seedlings after frost treatments as outlined in table 2. Results are based on one flat per treatment of approximately 50 seedlings of *Eju* and *Acr* from 1945 seed. Survival was recorded 5–7 days after treatment except for 69-day-old plants not until 16 days after treatment.

The seedlings of *Eju* were in all cases equal to or better than those of *Acr* in frost resistance; in six tests out of eight

in which there were survivors, *Eju* seedlings were definitely superior to *Acr* seedlings in resistance or in recovery after freezing. It is of interest in a comparison of treatments A and E that the 33-day-old seedlings showed the best survival. Percentage survival was lower with the advance in age to 55 and 69 days. Treatment A indicated that seedlings at 17 days were not so resistant as those several weeks older. A comparison of treatments A and E also indicates that recovery was equal or better in plants returned outdoors after freezing and without a thaw period at cool temperatures.

In addition to the role of seedling age, it is recognized that the results obtained may also be partially an expression of environmental conditions preceding and following each freeze experiment. The increase in mean outdoor temperature as the seedlings matured, with the accompanying increased plant growth, may have been in part responsible for the loss in frost hardiness of the youngest seedlings.

Drought resistance of seedlings

The northern Great Plains area frequently has periods of drought which may be of prime importance in the balance of climatic conditions which determines the success or failure of artificial or natural grass seeding. Any difference in the ability of these two important grasses to resist drought is of interest, therefore, especially in the seedling stage —the critical growth period. Mueller and Weaver (6), in studying the relative drought resistance of seedlings of some dominant prairie grasses under artificial drought tests, found that the results correlated well with the characteristics of the species under field conditions. The property of drought resistance has been variously defined, but, in any case, it includes the ability of the plant to survive a period of moisture deficiency resulting from either edaphic or atmospheric drought.

An experiment was started in August in a whitewashed greenhouse, using a loamy sand from the Anoka Sand Plain north of Minneapolis. The sand had been heat-sterilized to prevent growth of weed and other seeds included. Eight seedlings of each species were grown in 330-cc. culture cans, the soil covered with fine quartz sand to reduce evaporation. Each species and treatment was represented by two replicate sets of three cans each, placed in a randomized arrangement. The cans were insulated to prevent rapid temperature changes which might result in water of condensation and make water available which would otherwise be nonavailable.

All cultures were started with favorable soil moisture. In treatment A (table 4) no water was added after planting the seeds, and measurements of soil moisture and growth of seedling roots and shoots were made at the time of permanent wilting. The latter was defined as follows: both species exhibited at a definite time in their wilting a stage when the leaves became hard in texture and if bent at the base would remain stiff rather than being flexible; there was then also a characteristic hard and abnormal feeling to the leaves. No culture was classed as wilted until all the leaves of all five plants were in this condition. In treatment B (table 4) the soil was kept in good condition for growth for 25 days, at which time half of the cultures (B-1) were examined for soil moisture and length and weight of shoots and roots. The other half (B-2) were left unwatered until permanent wilting and were then similarly examined.

In all other treatments (C, D, E, and F) the plants were watered for 25 days, as in B, and left unwatered until permanently wilted. The variation among these treatments was in the time for which plants were left in the wilted condition—1, 3, 5, and 10 days, respectively. Following the period of wilting each treatment was watered according to a definite and uniform schedule. Four weeks after resumption of watering, measurements were made of the number of plants recovering, area and dry weight of the living, green leaf portions, and weight of the dead leaf parts.

The results from hygrothermograph records during this experiment showed the mean daily humidities and mean daily temperatures to be higher than during a comparable period of the spring

TABLE 4

SUMMARY OF RESULTS OF DROUGHT-RESISTANCE EXPERIMENT
(Replications consisting of 3 culture cans, five plants in each.)

Treatment	Species	Days drought to permanent wilting	Shoot ht. (cm.)	Shoot dry* wt. (mg.)	Root lgth. (cm.)	Root dry wt. (mg.)	No. plants* recovering (total 15)	Total green* leaf area (sq. cm.)	Total wt.† green leaves (mg.)	Permanent wilting percentage
A	Eju	36	9.4	16	12.5	9	.....	.....	.....	0.85
		34	11.6	13	12.0	11	.....	.....	.....	0.91
	Acr	36	13.7	23	14.4	10	.....	.....	.....	1.15
		33	17.2	20	11.2	13	.....	.....	.....	1.04
B_1	Eju	.....	14.7	37	15.5	23	.....	.....	.....	(6.69)‡
	Acr	.....	22.5	50	15.5	17	.....	.....	.....	(5.81)‡
B_2	Eju	24	13.1	34	18.3	26	.....	.....	.....	0.81
	Acr	16	23.1	65	13.8	26	.....	.....	.....	0.88
C	Eju	21	.....	77	.....	.....	10	12.1	22.0	.....
		22	.....	83	.....	.....	14	15.7	25.5	.....
	Acr	17	.....	143	.....	.....	5	6.8	11.0	.....
		19	.....	177	.....	.....	2	3.6	5.5	.....
D	Eju	22	.....	83	.....	.....	2	1.2	1.0	.....
		22	.....	89	.....	.....	0	0	0	.....
	Acr	17	.....	152	.....	.....	0	0	0	.....
		17	.....	148	.....	.....	0	0	0	.....
E	Eju	22	.....	81	.....	.....	6	2.9	3.5	.....
		23	.....	90	.....	.....	2	1.2	1.5	.....
	Acr	19	.....	117	.....	.....	0	0	0	.....
		17	.....	146	.....	.....	0	0	0	.....
F	Eju	22	.....	92	.....	.....	1	0.1	0.5	.....
		21	.....	100	.....	.....	0	0	0	.....
	Acr	19	.....	129	.....	.....	0	0	0	.....
		18	.....	146	.....	.....	0	0	0	.....

* Data for C–F at 28 days after the end of each drought period on the basis of fifteen plants in each replication.
† Weight of green leaves on basis of fifteen plants, not included in column of shoot dry weight.
‡ Soil moisture percentage on September 13, at end of 25-day watering period.

growing season in the northern Great Plains area, i.e., Mandan, North Dakota. The similarity of the evaporativity of the air was suggested by 14.7 inches water loss from small open-pan evaporimeters in this experiment compared to an average of 14.9 inches from large evaporimeters at Mandan.

The number of days of drought to permanent wilting in treatment A (table 4) represents the total time from planting to permanent wilting, no water having been added. The figure for each replication represents the combined results of three culture cans containing five plants each. The length of time from planting to permanent wilting in treatment A was only slightly longer for *Eju* than for *Acr*. The time period between the last watering at 25 days from planting until permanent wilting occurred (treatments B–F) reveals a greater contrast between the species. The average length of the drought period before wilting for all these treatments was 22.3 days for *Eju* in contrast to 17.5 days for *Acr*. This time difference of 5 days is significant, and the relative difference was consistent in each replication. Part of the explanation of this difference was apparent at the end of the 25-day watering period on September 13 when the samples of treatment B_1 were taken. At this growth stage of the seedlings—until then supplied equally with water—the soil moisture was higher for *Eju* than for *Acr* (B_1, table 4). A plausible explanation of this difference is seen by examination of the shoot height and weight as an indication of the amount of transpiring surface. Height measurements and root lengths are averages taken from five plants in each of three cultures. Oven-dry weights of shoots and roots represent the average combined weight of five seedlings per culture can. The 25-day-old *Eju* seedlings were only 65% as tall as those of *Acr* and the shoots only 74% as great in oven-dry weight. It is of interest, however, that the root growth of *Eju*, although equal in length, surpassed that of *Acr* by 33% in oven-dry weight. This greater root development of 25-day-old seedlings of *Eju* occurred when conditions were favorable. When the soil moisture was allowed to decrease, however, from after planting until the state of permanent wilting was reached, the root growth of *Eju* was no greater, or even less, than that of *Acr*, and the shoot growth was distinctly less for *Eju*, as indicated by the results of treatment A. Because of the lower ratio of shoot to root in *Eju*, the seedlings took considerably longer to reach the state of permanent wilting (table 4). It is of interest to compare the average permanent wilting percentages of 0.88 for *Eju* and 1.10 for *Acr*, arrived at empirically by growing plants in the soil until wilted, with the wilting-coefficient value of 2.5 calculated by multiplying the moisture equivalent of the same soil by the conversion factor often employed.

Not only is the duration of time which seedlings will endure a period of decreasing moisture important, but also the length of time which they will endure being in a wilted condition and still recover upon a renewal of soil moisture. In order to test *Eju* and *Acr* for differences in this regard, treatments C, D, E, and F were set up with periods of wilting of 1, 3, 5, and 10 days, respectively.

The length of time it took for the seedlings to reach the state of permanent wilting, once watering was stopped, was almost 5 days longer for *Eju* than for *Acr*. After remaining wilted for the designated period, the plants were watered at the same rate for 28 days. At the end of this period some *Eju* seedlings

had recovered in each of the few treatments, while those of *Acr* showed complete lack of recovery in all treatments except in C, where the plants were wilted only 1 day. Even in C an average of only three and one-half *Acr* plants out of fifteen recovered, in contrast to an average of twelve out of fifteen for *Eju*. There is, of course, the possible objection that there may be a difference in leaf structure which would cause some variation in the determination of the point of permanent wilting, which might favor *Eju*, but in all cases each replication of three cans of *Eju* was designated as being permanently wilted at a later date than those of *Acr*. This would indicate that if any difference in the period of wilting did exist, it was probably actually greater for *Eju* than for *Acr*. The plants were considered to be recovering if one or more leaves were green and evidently alive.

The area of the living green portions of the leaves (table 4) was calculated by multiplying the length and width of the green portion of each leaf. The areas given in square centimeters are the totals for the fifteen plants of three culture cans in each replication, as is the case also for the total weights of the green parts which were separated from the dead leaf portions. The total recovered green leaf area of all plants of treatments C–F was 208% greater for *Eju* than for *Acr*. The total oven-dry weight of the green leaf portions was 227% greater for *Eju*.

Among plants which recovered there was some variation in the development of leaves between the two species. In *Eju*, 65% of the green leaves of the recovered plants were newly formed; in *Acr*, only 37% were newly formed, the rest being regrowth of the leaf bases.

Attention is again directed to the greater shoot growth of *Acr* (shoot dry weight, table 4), while root growth was nearly equal. The greater amount of foliage of *Acr* would serve as an evaporating surface during the period of wilting and would be more effective in draining the moisture from its roots than would the lesser foliage of *Eju*. This more favorable shoot-root ratio of *Eju* apparently enables it better to retain its viability and food supply necessary for the initiation of new growth when favorable moisture conditions are renewed.

Bunker Prairie field experiment

To simulate the dry plains conditions where these species are grown extensively, an area of soil of xeric nature—Bunker Prairie in the Anoka Sand Plain, 3½ miles west of Johnsville, Minnesota—was selected. The combination of the sandy soil with the high temperatures and drought periods of the summer of 1947 proved to be extremely xeric. Four sites of varying topography and soil moisture were selected, and rectangular plots 4 × 20 feet were planted to each of the two species. These were aligned on the contour of the slope, and each plot was spaded to simulate disking and harrowing. Planting was done by hand with the use of a planting-board templet, individual seed being planted ¼ inch deep and 6 inches apart in rows spaced 6 inches apart. Position of seeds was marked with toothpicks to aid in future counting.

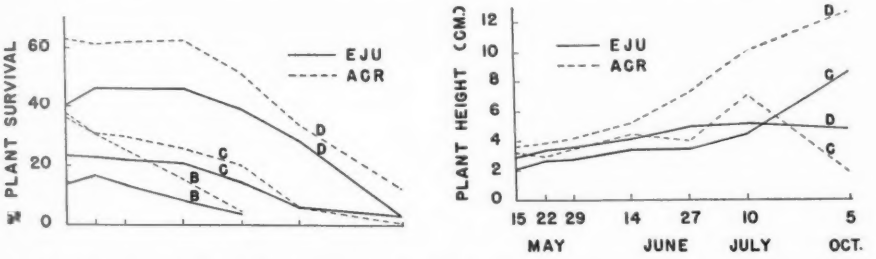
Of the four sites selected, the one at the top of a dune, Station A, had to be abandoned because of the extreme drought. Station B was located halfway down the south-facing slope from Station A, the dominant grass being porcupine grass, *Stipa spartea*. Station C was located at the base of the same dune and covered predominantly by Kentucky

bluegrass, *Poa pratensis*. Station D was located near by, with topography and soil like Station C, but had been plowed and then abandoned for over a year.

Analysis of a composite soil sample from Stations B, C, and D by the Soils Department, University of Minnesota, revealed a moisture equivalent of 4.6%, from which was calculated a wilting coefficient of 2.5%. Organic matter, nitrogen, phosphorus, and potassium were low in content. Measurements of atmospheric evaporation and temperature, soil temperature, and soil moisture were taken throughout the season, all of which revealed conspicuous summer drought and excessive temperatures, especially in August and October.

On seven dates during 1947 the growth and survival of the seedlings were checked. Earliest observations were made 17 days after the seeding on April 28. Each time a record was made of the number of plants living out of the possible 216 seeds planted, height of each individual plant, number of leaves per plant, observations of drought injury, insect damage, and seedling death.

The variation in percentage of surviving seedlings at each station is to be noted, being lowest for Station B, where the soil dried out most rapidly (fig. 7). Throughout most of the growing season the number of *Acr* seedlings was superior to that of *Eju*. This result is contrary to the indications from all previous experimental data including the standard germination tests, germination tests at various soil-moisture percentages, and seedling counts made in the drought-resistance study using soil taken from Station D of this experiment. At all stations, however, the rate of seedling mortality of *Acr* was surprisingly rapid. At Station B the initial number of seedlings of *Acr* was double that of *Eju*, but by June 27 the numbers were essentially equal. Likewise, at Station C the percentage of *Acr* seedlings on May 15 was 13.5% greater than for *Eju*, but on October 5 the number of *Acr* seedlings was 2% less. At Station D the superiority of number of *Acr* seedlings decreased from an advantage over *Eju* of 22% to that of 10%. As an average of the three stations the percentage germination and growth of seedlings to May 15 was 25.6% for *Eju* and 45.5% for *Acr*. On the last day of observation, June 27 for Station B and October 5 for Stations C and D, the average percentage of seedlings alive on the basis of number of seeds planted was 2.8% for *Eju* and 5.4% for *Acr*. On the basis of the number of seedlings present



FIGS. 7, 8.—Fig. 7 (*left*), seedling survival at Bunker Prairie, Minnesota. Planting was made on April 28. Percentages of survival are based on 216 seeds per plot. Station B, halfway up south-facing slope; C, base of same slope; D, comparable to C in abandoned field plowed 1 year before. Fig. 8 (*right*), average plant height at Bunker Prairie. Stations D and C are same as in fig. 7. Heights are based on measurements of every plant within plots at dates indicated.

on May 15, there was a survival at the last observation of 10.9% for *Eju* and 11.9% for *Acr*.

The foliage production of the two species is indicated by the curves for average plant height and number of leaves in figure 8. In addition to the normal drying back of some leaf tips, which alone may be enough to cause a reduction in plant heights with increasing age, there was some decrease in height owing to the feeding of a small population of grasshoppers. This damage, of course, upsets the attainment of a normal growth curve. Observations of the manner of damage by insects indicated no selectivity between the two grasses. The curves for height growth do bear out previous experimental results in showing that *Acr* produces a greater number of leaves than does *Eju*. The decline of the curve for *Acr* at Station C on the last observation can be credited to grasshopper damage on the single plant which had remained alive within the plot to that date.

Discussion

Both grass species are well adapted to the northern Great Plains with its extremes of temperature and moisture. Slight advantages in the timing of germination and seedling establishment of a few days may mean the difference between success and failure in seedings under unfavorable conditions. In the several experiments under a variety of moisture conditions, *Eju* seeds surpassed those of *Acr* in rapidity of water imbibition and germination. The germination and seedling establishment of *Eju* also surpassed *Acr* over a range of temperature conditions, although both grasses are generally favored by cool temperatures. Because of low temperature germination and rapid seedling growth, both species are suited to late-fall or early-spring seeding. The root growth of *Eju* was superior under favorable moisture conditions but was surpassed by the root development of *Acr* under the stress of limited soil moisture. At all times during seedling growth the foliage production of *Eju* was inferior to *Acr*, the plants being of shorter stature and of fewer leaves. However, *Eju* is more drought- and frost-resistant with the result that mature stands equal or surpass *Acr* in forage production.

Summary

1. Various phases of seed germination, seedling growth, and frost and drought resistance were compared for Standard crested wheatgrass (*Agropyron cristatum* [L.] Gaertn.) and for Russian wild-rye (*Elymus junceus* Fisch.).

2. Germination tests according to standard seed laboratory procedure indicate high percentages for both species, with an advantage for Russian wild-rye. The best results for both species were obtained by a 6° C. chill followed by a constant temperature of 20° C., or an alternation of 20°–30° C. without a chill.

3. The results of a series of germination tests are given at temperatures of 5° intervals from 5° to 30° C. based on readings at 6-hour intervals. The pregermination period for both species was shortest at 30° C. In all trials, 5° C. was the least favorable temperature for germination, and 30° C. was generally the second least favorable. The optimum temperature for Russian wild-rye was 25° C., both for rate of increase in germination and for percentage of final germination, while for crested wheatgrass the optimum after the first 10 days was in the range of 15° to 20° C., showing a greater adaptability of the latter species to germination at cool temperatures of early spring or late fall. This was especially true in

seed recently harvested, which was favored by cool germination temperatures.

4. Seed of Russian wild-rye imbibed water more slowly up to 48 hours, at which time the percentage of increased weight was about equal, but the number of germinated seeds was double that of crested wheatgrass.

5. In an experiment to determine the comparative germinating ability of the two species with different substrate moistures, it was shown that no seeds germinated at 0.5% moisture in fine quartz sand having a hygroscopic moisture coefficient of 0.1%. At all other sand moisture contents (1.6%, 3.4%, 8.2%, and 22.0%) Russian wild-rye was superior to crested wheatgrass in earliness and rate of increase of germination which always resulted in a higher final percentage of germination for Russian wild-rye.

6. A study to determine the comparative seedling frost resistance of the two species employed a total of five different freezing procedures on seedlings of four different ages. Seedlings of Russian wild-rye up to 10 weeks of age have a slower development of leaves, are shorter in stature, but are less susceptible to frost injury, at least throughout the first 8 weeks of growth, than are the seedlings of crested wheatgrass.

7. In a seedling drought-resistance study each treatment and species was represented by six cans of five plants each. When the soil moisture was favorable, Russian wild-rye seedlings produced a greater root system but less foliage growth in height, number of leaves, and oven-dry weight. During periods of unfavorable soil moisture, the root growth of Russian wild-rye slowed down and was equaled by that of crested wheatgrass at the time of permanent wilting. The ratio of leaves to roots was lower for Russian wild-rye than for crested wheatgrass, which may be important in drought resistance. Some plants of Russian wild-rye recovered when watered after periods of 1, 3, 5, and 10 days at permanent wilting, while no crested wheatgrass seedlings recovered which had been wilted for more than 1 day. Of the Russian wild-rye plants which recovered, most of the leaves were newly formed, while in crested wheatgrass they consisted mainly of extended leaf bases. In spite of the smaller amount of original foliage, the photosynthetic area of the recovered Russian wild-rye plants exceeded that of crested wheatgrass by more than three times.

8. Under field conditions, in the xeric environment of a sand-dune area, crested wheatgrass produced a greater number of seedlings and more foliage per plant as measured by number of leaves and plant height than did Russian wild-rye. But, during the growing season in which there was a period of hot, dry weather the seedling mortality of crested wheatgrass was so high that by fall the number of living seedlings was nearly equal to that of Russian wild-rye.

This is a portion of a thesis accepted as partial fulfilment of the requirements for the degree of Doctor of Philosophy in the Graduate School, University of Minnesota, in 1948. The writer acknowledges the suggestions and assistance of Dr. E. A. HELGESON and Dr. D. B. LAWRENCE and especially is grateful to Mr. GEORGE ROGLER for his many suggestions, information, and supplying of seed material. Appreciation is also extended to co-operative members of the Agronomy and Entomology Departments of the University of Minnesota Farm Campus, St. Paul, Minnesota.

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EXPERIMENTS ON PHOTOPERIODISM, DORMANCY, AND LEAF AGE AND ABSCISSION IN SUGAR MAPLE[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY **623**

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Introduction

The experiments reported below, carried on in the University of Chicago greenhouses and garden from 1941 through 1947, were initiated as part of a continuing study on the ecological life-history of sugar maple (*Acer saccharum* Marsh.). They deal primarily with the influence of photoperiod and, in part, of temperature on the gross processes of leaf senescence and abscission (and hence on leaf age), of bud inhibition, the rest period, and breaking of dormancy, and on the duration and amount of vegetative growth. The results have a bearing on experimentation with and interpretation of the internal factors which basically control these processes, as well as specifically on this species and on the purely ecological aspects of these phenomena. Some of the data can be interpreted in terms of modern concepts and theories of hormonal control of these processes (**3, 7, 14, 18, 20, 21, 23, 29, 30, 31, 36, 37, 38, 43**), but some also raise certain questions regarding the details of such control, and especially as to the nature of the action of environmental factors on the physiological mechanisms.

The influence of photoperiod on various vegetative phenomena in temperate-

[1] This work was aided in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

zone woody plants has been investigated sporadically since such effects were reported by GARNER and ALLARD (19). No attempt will be made to cite or review comprehensively all recorded observations and experimental work, since the subject has been recently reviewed by WAREING (40). Extension of the photoperiod beyond natural daylength may result, when comparing woody plants on long with those on natural or shorter day, in one or more of the following effects: (*a*) Delay in senescence and abscission of leaves but usually not complete inhibition of these processes (19, 27, 32, 40), although in some species continuous light or a very long photoperiod apparently does inhibit abscission indefinitely (19, 27, 39). (*b*) Limited or no acceleration in completion of the rest period by dormant buds (1, 19, 43), or in some species a weak or marked stimulation to renew growth (19, 22, 28, 34, 35, 40, 41), presumably in many cases when buds were not in deep rest. (*c*) Prolonged continuation of terminal growth once started (19, 27, 28, 34, 35, 40, 41), or a stimulation to repeated flushes of growth (22, 43), or, rarely, earlier cessation of growth than on natural or short day (19), or essentially no effect on duration of growth period where leaf and node number are morphologically determined the preceding year (42). (*d*) Greater length of stems, either through an effect on prolonging growth (19, 22, 27, 28, 35, 39, 41) or on elongation of individual internodes (41, 42). (*e*) Prolongation of cambial activity (43). (*f*) Decrease in frost resistance (34). Effects of shortening the photoperiod below natural daylength are usually the converse of those resulting from its extension and need not be reviewed specifically.

It has been assumed from the results of most of the work cited above that photoperiodic control may be important in periodic phenomena under natural conditions for the species investigated; but there has been little conclusive evidence that these effects are of actual and major ecological and geographical significance for native woody plants. Experimental photoperiods employed have often been extremes as compared with those occurring naturally in those seasons when such effects would be important for the species investigated, have usually involved constant photoperiods, and have not been studied in interaction with various levels of other factors. Similar limitations as ecological experimentation obtain in results reported here. Most of the data amassed to date are therefore more meaningful in conjunction with physiological interpretations of the internal mechanisms controlling these periodic processes which in nature are obviously multiconditioned. WAREING (43) has recently elaborated a hypothesis on the mechanism of photoperiodism in *Pinus silvestris* involving the view that two mutually antagonistic systems operate within the plant: (*a*) one promoting continued shoot growth, which depends upon some substance produced during the light period, and (*b*) an inhibitor-system which becomes operative under 4 hours of dark, and which results in earlier cessation of growth in proportion to the length of the dark period. The photoperiodic effects in sugar maple reported below have some bearing on this hypothesis.

Since the pioneering work of LAIBACH (29) and LA RUE (30), the extensive work on experimental and horticultural control of leaf and fruit abscission by means of naturally occurring or synthetic substances (2, 18, 20, 21, 31) has led to a widespread acceptance that the petiole is provided by its leaf blade with

an anti-abscission auxin and that presumably abscission under natural conditions proceeds when the rates of production and/or translocation of such a naturally occurring inhibitor(s) in aging leaf or fruit drop below a certain minimum, thereby lowering its concentration at the potential regions of abscission. The physiology of leaf abscission itself, however, as contrasted with that of debladed petioles (**7, 13, 16, 21, 31**), has not been extensively investigated. Many data on induced or inhibited abscission are easily interpreted through such a concept, as are also many observations on premature or retarded defoliation and flower and fruit abscission as apparent responses to the interacting influences of natural environmental factors—including length of photoperiod—and aging. The mode of action of such environmental influences, however, on production, translocation, or inactivation of such an inhibitor and especially the nature of its inhibiting action in the abscission region are still obscure, as is also the mechanism of localization of the abscission response in morphological development (**17, 21**). GAWADI and AVERY (**21**) have recently itemized the agents, natural and artificial, known to accelerate or retard abscission. They have also tentatively proposed a hypothesis that abscission behavior is a matter of relative unbalance between two substances—one an auxin-like substance instrumental in inhibiting abscission while leaves are young but diminishing in supply as the leaf ages, the other ethylene, or a substance with similar physiological effects, which is produced in mature tissues and which accelerates aging. SHOJI *et al.* (**36**) in experiments with Black Valentine beans have found an auxin gradient across the abscission zones of leaflets when the leaf is active; this gradient is lost immediately preceding abscission. They also mentioned other experiments, to be reported later, indicating that "the auxin gradient, rather than the absolute amount of auxin, controls abscission."

There is less agreement on what happens in the buds of temperate-zone woody plants during the rest period that precedes their renewal of growth following adequate chilling (**3, 11, 13, 18, 23, 24, 25, 26, 37, 38, 44**). Chemical or physical agents—including lengthened photoperiod—have been used in numerous ways to "force" such resting buds (**2, 3, 6, 11, 12, 14, 15, 24, 25, 26, 33**), and certain applied chemicals may delay budbreak (**2, 12, 18**). These facts have often been interpreted on the basis of destruction, diminution, or augmentation of specific growth inhibitors or activators (including auxin) and their relative balance. But in spite of this work little light has yet been shed on the mechanism of the action of cold under natural conditions on either hypothetical or demonstrated changes which take place in the buds. WENT (**44**) has stated that it is impossible to suggest a hypothesis as to how low temperatures could remove inhibitors or lead to synthesis of growth-promoting substances. CURTIS and CLARK (**13**) pointed out the possibility of a dissimilarity between the breaking of rest by injurious reagents and the more natural termination under conditions of low temperature.

Much of the work and many of the reviews (**3, 4, 5, 8, 9, 10, 11, 12, 13, 25, 26, 44**) involving factual description of chilling requirements for completion of bud rest have tended to emphasize the necessity of exposure to temperatures within a certain range for a minimum number of hours. Thus, WENT stated (**44**) that only temperature values below

5°–8° C. seem effective in breaking dormancy and that these low temperatures must last for a sufficient number of hours for completion of rest. He implied that a minimal number of hours below 5° C. could be determined for this requirement in each species and variety. CURTIS and CLARK (13) indicated an effective temperature range from 0° to 6° C., and CROCKER (12) that temperatures between 0° and 10° C. were effective but that high temperatures and temperatures below freezing were not. AVERY and JOHNSON (2) quoted W. H. CHANDLER (from correspondence), however, that the degree to which bud rest is completed could not be measured accurately by the number of hours of exposure below 45° F. or by any other published temperature measure, since the effect of chilling is modified by the past history of growth in the preceding summer and by the nature of the autumn. CHANDLER (9), citing work by BENNETT, stated also that, when temperatures during chilling fluctuate to a maximum of about 70° F., buds not only make no advance toward completing their rest during the warm phase but are pushed back deeper into it. BENNETT (4, 5) and CHANDLER (8, 9) have both stressed that bud and twig temperatures, not air temperature, are the decisive values in such studies.

Almost no specific data for sugar maple have been found which bear on these problems. ADAMS (1) reported essentially no effect of continuous light in inducing dormant buds of this species to grow any earlier than on natural daylength. He concluded that "the dormant habit in sugar maple must be very deep-seated." No critical photoperiodic studies on the species seem to have been reported, but several other species in the genus have been tested. GARNER and ALLARD (19) reported that small individuals of *A. negundo* and *A. rubrum*, exposed to an 8-hour photoperiod beginning early in January, successfully unfolded their leaves at about the same time as the controls but made little subsequent growth even under a 10-hour day. They illustrated an extreme effect in *A. negundo* from exposure to a 10-hour photoperiod as compared with natural daylength, the treatment beginning on May 2. On September 22 the plants on short photoperiod were 6–8 inches tall, with little persisting foliage, while those on natural day were about 2 feet tall and in full leaf. JESTER and KRAMER (27) found greatest height growth in *A. rubrum* with continuous light (under this condition it grew all winter in the greenhouse), more on long than on natural day, and more on natural than on short photoperiod. This species on continuous light in the greenhouse retained most of its leaves from its first growing period for the entire observation period of almost 2 years. With long photoperiods (not continuous light) it retained its leaves longer than on natural day. ODÉN (cited in 32) reported a prolongation of leaf-fall through the winter—instead of the usual rapid autumnal one—when *A. campestre* was placed on a 17-hour photoperiod in the autumn. MATZKE (32) found eight street trees of *A. platanoides* showing prolonged autumnal retention of leaves near electric lights but stated that numerous other examples exhibited no effect. Also, in *A. saccharinum* he found no convincing evidence of the effect of street lights on leaf retention. WAREING (43) referred to his unpublished data on *A. pseudoplatanus* which indicate that there is no critical photoperiod above which its growth may be maintained indefinitely and that its formation of resting buds is not appre-

ciably delayed, even under continuous light, as compared with their formation under natural long days.

BRAMBLE (6) reported that buds on 4-foot sugar-maple trees, brought into a warm greenhouse in early autumn before leaf-fall, were still dormant the following June, and that, although some of them became active later, their foliage was not yet fully developed in September. He found that their dormancy could be broken by treatment with ethylene chlorhydrin in early June, the trees leafing out in June and July. DEUBER and BOWEN (15) earlier had found that smaller sugar-maple trees brought into a warm greenhouse in October could not be forced satisfactorily by ethylene chlorhydrin treatment until January and that even then trees which had been kept outside were forced more readily than those kept inside. Trees forced in January had nearly full-sized leaves by March 15. This is a slow response and growth rate compared with many other species. HOWARD, in the first of his classical studies on winter rest (25), tested *Acer saccharinum* Wang. This was presumably *A. saccharum* Marsh., but he did not give the origin of his material. Stem cuttings brought inside at Columbia, Missouri, as controls for forcing treatments, failed to grow when collected over the interval October 30–February 12. Only one lot, collected between January 21 and February 2, could be forced by etherization, buds showing growth in 13–17 days. None was forced by his drying treatment. These few examples indicate that the species shows profound "rest," as suggested also by its field behavior.

Material and methods

The origin, history, and general care of the seedling population of sugar maple used in all experiments were as follows: On May 16, 1941, approximately five hundred newly germinated seedlings (cotyledons partially expanded) were collected at Wychwood, Lake Geneva, Wisconsin, from under two isolated but native seed trees in the gardens of this estate. Pollen parents of the population were probably numerous, since the gardens are surrounded by extensive forests in which this species is dominant. No *A. nigrum* Michx. f. occurs in them, and all seedlings were easily referable to *A. saccharum*. Morphological variation of genetic origin, except for size, was not marked at any time during the 7 years in which this seedling population was under observation, although part of the physiological variation within any one experimental series, as described later, was undoubtedly genetic and may be considered as a sample of the variability in response to be expected in natural seedling populations of this species.

The seedlings were brought to Chicago, planted singly in fertile loam soil in 3-inch unglazed clay pots, placed in flats in a cold frame until established, and then moved into the open in full light on natural daylength. During this time the two primary leaves expanded, and terminal winter buds were differentiated. In late June the plants were transplanted to 5-inch pots with similar soil in which they were left thereafter, with soil added as necessary to fill. Watering and general care followed normal greenhouse routine. Although the seedlings eventually became potbound, it is thought that this condition did not significantly affect the processes under observation.

Following the transplanting in June over half the seedlings renewed terminal shoot growth and expanded two or four more foliage leaves in July before resting buds again were formed. This second

flush may have been stimulated in part by root injury incident to transplanting. All seedlings received similar treatment until September 10, 1941.

Experimentation

Experiment I. Leaf senescence and abscission in 1941

The general objectives were to test the effects on the persistence of green leaves and on abscission of the 1941 foliage of the interactions of (*a*) photoperiod, (*b*) outdoor versus greenhouse environment, and (*c*) age of leaf.

Procedure.—On September 10, 1941, when natural photoperiods were still approximately 13 hours, the population (least vigorous plants were discarded) was divided into five series with experimental conditions and numbers of plants shown in table 1. Each series included subseries of plants which had made one or two flushes of growth, but terminal buds on all plants were then dormant and remained so until the experiment was terminated. Presumably these buds were already in deep rest; they were not visibly stimulated by the conditions employed. All leaves on plants of one flush and leaves of the first flush on plants which had grown twice were approximately 2 months older than those which had expanded in the second flush in July. The indoor series (II–V) were placed in one greenhouse room (minimum temperature 65°–70° F.) on movable trucks

TABLE 1

PERCENTAGES OF PLANTS STILL BEARING GREEN LEAVES

Series	No. plants	Photoperiod	Percentages on				
			Oct. 20	Nov. 13	Dec. 13	Jan. 8	Feb. 11
	Outside						
I*a* *	15	Natural	7	0	0	0	0
I*b* †	15	Natural	87	0	0	0	0
	Inside—one flush of growth						
II*a*	87	16–8‡	100	90	40	1	0
III*a*	30	16	100	90	43	20	3§
IV*a*	30	12	100	77	3	0	0
V*a*	30	8	100	30	0	0	0
	Inside—two flushes of growth						
II*b*	108	16–8‡	100	100	92	47	0
III*b*	40	16	100	100	97	87	55§
IV*b*	40	12	100	100	65	50	15§
V*b*	40	8	100	95	25	12	5§

* One flush of growth.
† Two flushes of growth.
‡ Photoperiod changed from 16 hours to 8 hours on December 13.
§ Values were zero on March 26.

which were daily rolled into and out of ventilated lightproof sheds for the control of photoperiod. Temperature differences among the various sheds were negligible. For series II, III, and IV natural daylight exposures of 8 hours (8:30 A.M.–4:30 P.M.) were supplemented after 4:30 P.M. by radiation (100–180 foot-candles at leaf surface) from incandescent-filament lamps in the sheds to provide the indicated photoperiod. Series V and, after December 13, series II received only the 8 hours of natural daylight. These conditions were maintained until February 11, 1942. It should be emphasized that the intensity values of the supplemental light employed here and at other times are almost inadequate for growth of sugar maple, although this general level of intensity allows survival of some seedlings for several years under natural forest conditions. Hence the photoperiodic effects reported are presumably largely "hormonal" in origin rather than related to variation in carbohydrate supply because of any differences in opportunities for photosynthesis.

Observations were made at periodic intervals on the percentages of plants showing (*a*) easily perceptible green color persisting in some leaves, (*b*) all leaves nongreen (usually brown but often containing enough anthocyanin to be reddish), and (*c*) all leaves abscised. Data are presented only for class *a* (table 1); this criterion of apparently functional versus nonfunctional leaves was less subjective than the distinction between classes *b* and *c*, since the actual time of fall of a completely nongreen leaf under greenhouse conditions was apparently conditioned by the chance occurrence of mechanical forces sufficiently strong to cause detachment. It was found that leaves possessing more than a few small patches of green color were not easily detached in the abscission zone even though the separation layer was apparently partly differentiated; in contrast, most nongreen leaves were easily detached by slight pressures but might persist on the plants several weeks if such pressures were not exerted. Data presented for plants with two flushes of growth are based on the status of the leaves of the second flush; their first-formed leaves showed behavior in time similar to that of leaves of plants of only one flush, indicating a leaf-age effect on senescence and abscission of individual leaves shown strikingly by the data presented for the two groups (table 1).

RESULTS.—In series I*a* (outdoors—one flush of growth) loss of green color and abscission in October occurred in the time sequence characteristic of trees of this species in the Chicago region. Loss of green color was practically complete by October 20, and abscission was total before November 1. No frosts had occurred. In series I*b* (outdoors—two flushes) leaves of the second flush remained green 7–10 days longer. No green leaves were recorded on November 1, and abscission was complete by November 13. These data suggest the importance of a leaf-age effect on senescence and abscission even in these plants outdoors.

An interaction between age of leaf and photoperiod in affecting senescence is brought out in the data for the indoor series II–V (table 1). Constant short photoperiods (8 hours, series V) hastened, long ones (16 hours, series III) delayed, the onset of senescence in each series as a whole in comparison with series IV (12 hours). An abrupt change in photoperiod from 16 to 8 hours accelerated loss of green color (series II versus series III). The age-of-leaf effect is striking in a comparison of subseries *a* and *b* in each series, loss of green color for each

subseries as a whole occurring much earlier in plants of one flush than in those of two. All plants inside (series II–V), irrespective of photoperiod or age of leaf, were delayed in leaf senescence in comparison with series I (outside). These series differed in both temperature and photoperiodic conditions, and no assumptions should be made as to the relative significance of these factors in the differential response. The desirability of running an indoor control series on decreasing natural photoperiod was not appreciated at the time this experiment was started.

It should be emphasized that these data reflect variation in response within each subseries presumably resulting from genetic variation; the percentages given represent those individuals still retaining green leaves on a given date; the remainder were nongreen or had abscised their leaves. That such variation within a subseries was primarily genetic in origin is suggested especially by such subseries as III*a* or III*b* in which some plants retained green leaves for 3–4 months longer than did others. It is difficult to relate such variation to a past-history factor, or to environmental variability within a subseries. The possibility of such genetic variation should be carefully considered in interpretation of the results of critical studies on the physiology of leaf senescence and abscission; but it might be used as a tool in such studies. It is interesting that in later greenhouse experiments such variation was least obvious in populations on natural photoperiods. Presumably the imposed conditions of constant photoperiods and warm temperatures in this experiment allowed greater phenotypic expression of the genotypic differences than do naturally decreasing photoperiods, either indoors or out.

CONCLUSIONS.—Abscission of leaves in sugar maple under the experimental conditions used is correlated with their loss of green color. The onset of this latter event is a function of chronological leaf age in relation to external environmental conditions, of which length of photoperiod is of significance. A 16-hour photoperiod imposed a month prior to normal leaf-fall retarded leaf senescence and abscission as compared with plants on shorter constant photoperiods but did not stimulate resting buds to break dormancy.

EXPERIMENT II. BUD-BREAK, GROWTH, AND ABSCISSION, 1942

The objectives were to test the effects of (*a*) past history—the variables of experiment I; (*b*) differential exposure of the dormant seedlings to cold; and (*c*) different photoperiods, as interacting factors conditioning (*x*) time and rate of renewal of growth from terminal buds, (*y*) its continuation and extent, and (*z*) persistence or abscission of leaves produced as a function of their age as well as of the other variables.

PROCEDURE.—On February 11, 1942, the 399 surviving plants of series II–V of experiment I were systematically reassorted into three new series (A, B, C) with numbers derived from the preceding series as indicated in table 2. An attempt was made for series A and B to establish populations parallel as to size range of plants and past treatment; series C was not completely so, since in it were included all plants which still bore green leaves on February 11 (table 1). All plants of these three series were returned to natural daylength on that date except those of series C with green leaves which continued on their respective photoperiods until March 26. All green color had then disappeared.

On February 11 series A was placed in a cold greenhouse room in which temperatures fluctuated between 38° and 61° F. until March 3. It was then put outdoors in a sheltered location but experienced the variable outdoor weather, including freezing temperatures, until April 3, when it was returned to a cool greenhouse (temperatures ranging from 40° to 70°). Series B remained in a warm greenhouse until March 3, was then placed in a cold house (temperature range from 41° to 65° F.) until March 18, and was then put outdoors and was subjected to cold and freezing until it was also returned to the cool greenhouse on April 3. Series C remained continuously at a temperature of 70° F. or above after February 11. Thus, the three series differed significantly in degree and duration of exposure to temperatures which have proved suitable for fulfilling the chilling requirement in completion of the rest period in many deciduous trees and shrubs native to cold temperate climates. No attempt has been made, however, to evaluate this difference in terms of hours of exposure to temperatures below a certain value, since this was unnecessary for the major purposes of the following experimentation. The results (fig. 1) are conclusive that the chilling treatments were adequate to reduce to a low level any internal factors inhibiting growth in most plants in series A but not in series B.

TABLE 2

NUMBERS OF PLANTS IN SERIES OF EXPERIMENT II DERIVED FROM EACH SUBSERIES OF EXPERIMENT I

Subseries in experiment I	Series in experiment II		
	A	B	C
II*a*	30	30	26
III*a*	10	10	10
IV*a*	10	10	10
V*a*	10	10	10
II*b*	30	30	44
III*b*	14	14	12
IV*b*	14	14	12
V*b*	14	14	11

On April 28 each series was approximately equally subdivided into three subseries (designated 9, 20, and N) exposed thereafter to daily photoperiods of 9 or 20 hours or natural daylength. Plants representing the range of size variation, in flushes of growth in 1941, and in previous photoperiodic treatment were distributed equally, so far as possible, among the three subseries in each of the three series. If those exposed to different photoperiods in 1941 are combined, the numbers in each series with one or two flushes of growth in 1941 were as shown in table 3. Each of these eighteen populations included from two to fifteen (in most cases four, five, or ten) plants from each of series II, III, IV, and

TABLE 3

NUMBERS OF PLANTS IN SUBSERIES OF EXPERIMENT II SHOWING ONE OR TWO FLUSHES OF GROWTH IN 1941

Subseries	Series		
	A	B	C
	One flush, 1941		
9	22	22	21
20	22	22	21
N	16	16	14
	Two flushes, 1941		
9	25	25	27
20	25	25	27
N	22	22	25

V of experiment I, and the ensuing performances of the seventy-two resulting subpopulations were recorded separately.

Subseries 9, 20, and N were placed in separate greenhouse rooms approximately similar in conditions of natural light intensity and temperature (minimum temperature 70° F.). Subseries 9 was daily exposed to 9 hours of natural daylight only. Subseries 20 was on an open bench on natural daylength with supplemental illumination (less than 100 foot-candles from incandescent-filament lamps) in the evening to extend the photoperiod to 20 hours until October 15, then on a movable truck with 9 hours of natural daylight in the greenhouse and 11 hours of artificial light in a photoperiodic shed until February 3, 1943, and then on 8 hours of natural daylight only until March 12, 1943. The 9 and 20-hour photoperiods were selected for convenience and as extremes above and below those naturally encountered by sugar maple anywhere during vegetative activity and hence likely to affect significantly the renewal of growth, its continuation and extent, and persistence of leaves or abscission. The contrast in the results between series I and the other series in 1941 indicated the desirability of maintaining a series on natural daylength. This experiment was terminated on March 12, 1943.

Beginning on May 14, 1942, observations were made at weekly or biweekly intervals (in a few cases less often if little change was occurring) until March 12, 1943, on the numbers and percentages of plants in each of the seventy-two subpopulations which showed the following conditions: (*a*) still dormant, as indicated by no obvious swelling of buds; (*b*) one or more expanding buds; (*c*) in full leaf from first flush; (*d*) second flush of terminal growth; (*e*) badly injured through infection or other injuries; (*f*) dead through any cause; (*g*) loss of green color in most leaves; and (*h*) abscission of all leaves. Data on second flushes and senescence of leaves have been adjusted by eliminating from consideration plants injured or dead from causes extraneous to the experimental variables.

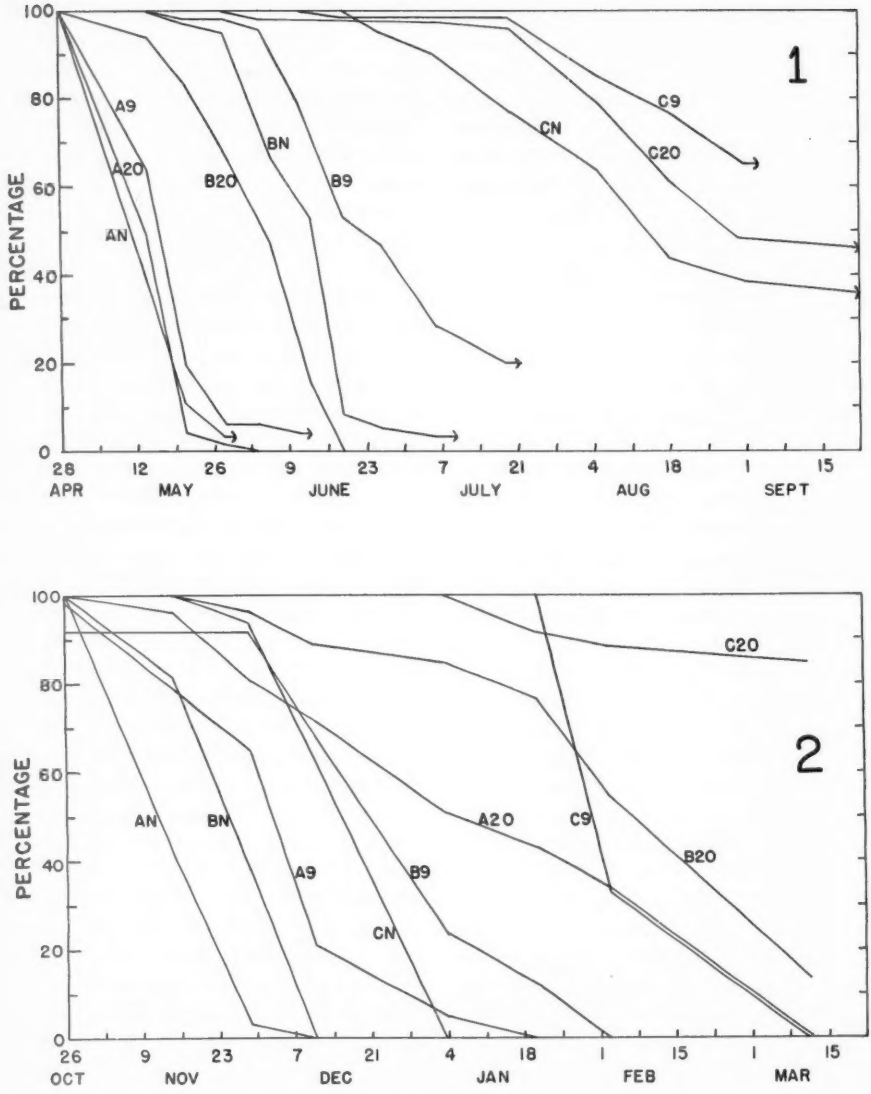
BUD-BREAK.—Extent of chilling was the dominant factor in reduction of internal inhibitory factors controlling renewal of growth from terminal buds, but its effects were modified in detail by the subsequent photoperiod to which the plants were subjected, the longer photoperiods (either N or 20) favoring growth, even in unchilled plants, in contrast with the 9-hour one (fig. 1). Swelling of buds in some plants of series A (most chilling) was evident by May 1. By May 14 half the plants in this series showed such swelling (or expanding leaves), with a greater percentage on 20-hour or natural than on 9-hour photoperiod. Four plants of this series then had fully expanded leaves. The 50% level of breaking dormancy (50% of plants with swollen buds or expanded leaves) in series B (intermediate chilling) was not attained until June and appeared successively later in subseries 20, N, and 9, respectively. In series C (no chilling) this 50% level was attained in August and September by plants of subseries N and 20, but only 35% of the plants of series C on 9-hour photoperiod broke dormancy during the entire experiment. Thus, reduction in levels of internal factors inhibitory to terminal growth was affected both by current photoperiodic conditions and ultimately by aging, in addition to the dominant role played by chilling.

The time of growth renewal varied within each of the nine subseries and showed greater range with intermediate or no chilling than with longer chilling

(as shown by the slopes of curves in figure 1). This should be noted in relation to presumed differences in levels of inhibitory factors as conditioned by past history prior to chilling. Contrary to expectation, it was impossible to establish any correlation between this range of variation in any one of the nine subseries and the past history of its subpopulations composed of plants showing one or two flushes of growth in 1941 or exposed to different photoperiods prior to chilling. Because of the findings of CHANDLER *et al.* (**10**) and others, it had been surmised that, within each subseries, plants on which green leaves from 1941 had persisted longest might be relatively more delayed in renewing growth than those which abscised earliest. Such was not the case. Within any one subseries renewal of growth might occur earlier or as early in some plants which showed two flushes of growth and were subjected to 16-hour photoperiod to delay leaf senescence in experiment I than in plants of one flush subjected to 8-hour photoperiod. This suggests that the photoperiodic conditioning of time of abscission did not consistently affect depth of bud rest. Since correlations between past history prior to chilling and time of renewal of growth could not be established, genetic differences are suggested as a factor in the variation in bud rest within a subseries, although with less certainty than in the variation in time of leaf senescence in each subseries in experiment I where all plants of a subseries had received prior identical treatment and the populations were larger. That such genetic variation exists among nonclonal populations of subspecific level in many horticultural forms is well known (**8, 10**); it is commonly employed as a basis for acclimatization of certain species to areas of mild winter climate (**8, 24**). The plants in series C which renewed growth without chilling, especially on the shortest photoperiod, must have differed significantly in depth of bud rest resulting from genetic differences from those which remained dormant.

GROWTH RATE AND SURVIVAL.—The rate of unfolding of new shoots, once bud swelling was detected, was somewhat positively related to degree of previous chilling, but no careful analysis of these data has been made. It has been reported repeatedly that, even when they proceed, the rate of growth processes in comparable species may be limited as a consequence of insufficient chilling. This limitation was most obvious in those plants of series C (no chilling) which eventually came into leaf. Expansion was slow, and size of leaves was below average. Many plants on 9-hour photoperiod, even those which were chilled, also grew slowly. Growth was most rapid and vigorous in subseries A20 (maximum chilling and longest photoperiod).

There was also a correlation of degree of chilling and current photoperiodic treatment with survival of those plants renewing growth. Of the 168 plants which broke dormancy from series A20, AN, B20, and BN (total 170 somewhat chilled and on long or natural photoperiod), all but 2 completed their first flush of growth; of the 83 breaking dormancy from series A9 and B9 (total 97 chilled and on 9-hour photoperiod), 13 died before attaining full leaf; in series C20 (total 48 unchilled and on long photoperiod), 27 of 28 breaking dormancy survived; in series CN (total 39 unchilled and on natural photoperiod), 18 of 25; and in series C9 (total 40 unchilled and on short photoperiod), 6 of 17. Thus, the shorter photoperiod and lack of chilling resulted not only in limiting the number



FIGS. 1–2.—Bud-break and leaf senescence in sugar maple in relation to previous chilling and photoperiod. Series *A*, most chilling; series *B*, intermediate chilling; series *C*, no chilling. Subseries *N*, *9*, and *20*, respectively, placed on natural, 9-hour, and 20-hour photoperiods after chilling (see text for details). Fig. 1, percentages of plants in each subseries still dormant on indicated dates. Arrows show final percentages remaining dormant (or dead). Fig. 2, percentages of plants still retaining one or more partially green leaves on indicated dates, based on 100% on plants within each subseries which broke dormancy by September 15 (fig. 1).

of plants which broke dormancy (as shown by swelling of buds), but many of those which started to grow died before attaining full leaf. Death in many cases resulted from injuries by insects or from "wilt" of unknown ctiology, but the incidence of such pathology was much greater in those subseries showing least vigorous growth.

SECOND FLUSH.—A most interesting result was the effect of different photoperiods on the number of flushes of growth. At the completion of the first flush all plants had partially formed new scaly terminal buds, presumably because of their morphological initiation preceding the first flush. These buds on plants on 9-hour photoperiod (subseries A9, B9, and C9) passed into rest without exception, and no plant made a second flush (the only case of uniformity in behavior within a subseries in this experimentation). On natural photoperiod (subseries AN, BN, and CN) 21 (23%) of the 90 plants completing a first flush made a second one; and on 20-hour photoperiod (subseries A20, B20, and C20) the comparable values were 85 (70%) out of 121. In practically all cases the second flush followed in from 3 to 8 weeks after completion of the first, irrespective of when the first occurred, but it was a little later on natural than on 20-hour photoperiod. Thus, the photoperiod in excess of natural was apparently responsible for this more general resumption of growth in the 20-hour subseries. It has been reported by others that terminal growth of some woody plants is prolonged on long photoperiod, but in the reports careful distinction has not always been made between continuation of a first flush and renewal of growth after resting buds were well formed. In the writer's experiment the second flush was soon terminated with formation of resting buds, which remained dormant thereafter until the following year, irrespective of the photoperiod.

Whether a plant made one or two flushes on 20-hour or natural photoperiod was not strongly correlated with its previous environmental history. Of the past-history variables, degree of previous chilling was of most significance. Based on all plants completing one flush as 100%, the percentages completing two flushes on natural photoperiod were 17, 29, and 22 for no, intermediate, and maximum chilling, respectively; on 20-hour photoperiod, 59, 79, and 68. Thus, for plants which made one flush no chilling was least conducive to two flushes, but intermediate chilling seemed more favorable than maximum. No explanation is suggested. No relation of photoperiodic effects in 1941 to number of flushes in 1942 could be detected. There was a limited relationship of the latter to number of flushes in 1941. On 20-hour photoperiod there was no effect, 70% of plants with either one or two flushes in 1941 showing two flushes in 1942; on natural photoperiod the percentages showing two flushes in 1942 were 17 and 27, respectively, for plants of one and two flushes in 1941.

SENESCENCE AND ABSCISSION.—In the autumn of 1942 loss of green color and abscission were observed and evaluated as in experiment I. It should be noted, however, that in experiment II the plants had been on the indicated photoperiods before coming into leaf, whereas in experiment I they were transferred to the experimental photoperiods from natural daylength about a month prior to the normal time for abscission. The data presented (fig. 2) are based on the number of living green plants in each subseries on September 15 as 100%. Each curve is based on from twenty-five to forty-seven

plants in all but subseries CN (16) and C9 (3). These data must be interpreted in light of the data on renewal of growth (fig. 1) and on number and dates of flushes of growth in the different subseries as affecting leaf age. To clarify the relative effects of current photoperiod versus age of leaf in affecting senescence, comparisons are best made among the three subseries of a series, or among the three subseries of similar photoperiod from the three series. Thus, most of the twenty-six plants of subseries C20 (series C was unchilled) remained green throughout the winter not only because of the long photoperiod but because of the youth of their leaves, which, for both flushes, were expanded chiefly in August, September, and October. Leaves on the three plants of subseries C9 became senescent sooner, even though mostly expanded in August and September, because only one flush of growth was represented, and senescence was accelerated by the short photoperiod. In a comparison of subseries A20 with A9 and B20 with B9 (fig. 2) the effect of the 20-hour photoperiod in delaying leaf senescence is related both to its effect on senescence per se (as demonstrated in experiment I) and to the large number of plants induced to make two flushes on the longer photoperiod versus none on the short one; persistence of the young leaves of the second flush accounts in part for the much more gradual slopes of the curves for subseries A20 and B20.

It is interesting that subseries CN became senescent earlier than C9, even though including some plants which made two flushes. Considering this last fact, its noteworthy acceleration in senescence over that of C9 can be most easily noted by a comparison of the curves for the two subseries in figures 1 and 2. Although CN completed its first flush somewhat before C9, this lead was much less than its lead in senescence, and it can be concluded that its individual leaves were shorter-lived than those of C9. Some of the data of experiments IV and V also confirm the following conclusion first tentatively arrived at on the basis of these and other results of experiment II: that a decreasing natural photoperiod probably accelerates senescence, even though its length may be longer than that of a constant one (in this case always more than, versus 9 hours), and that absolute leaf age is of less significance in determining the beginning of senescence when plants are on such a decreasing photoperiod than when on constant ones. The steepness of the curves for subseries AN, BN, and CN (all including some plants of two flushes) in contrast to those of A9, B9, and C9 (all plants of one flush only) support this conclusion, the populations on natural photoperiod exhibiting least variation in onset of senescence, even though some of their plants had relatively young leaves. Leaves of similar age on either 9- or 20-hour photoperiod persisted much longer.

The variation in time of senescence within a subseries, as shown by the slopes of curves in figure 2, is probably also related to genetic differences, as suggested in experiment I, but such variation here is so confounded with that resulting from past-history effects (especially one versus two flushes of growth and the actual time at which leaves expanded) as to preclude statements on its probable importance.

CONCLUSIONS.—Completion of the rest period in dormant buds of sugar maple under natural conditions is primarily the result of sufficient chilling. The process was accelerated after either some or no chilling by exposure to a photoperiod (20 hours) in excess of natural and was

somewhat retarded by a photoperiod (9 hours) shorter than a natural one. In some, but not all, unchilled plants aging alone in the dormant condition may result in the renewal of growth. It is possible that other environmental stimuli, such as summer heat, were effective in breaking dormancy in this latter case.

Once growth is renewed, a 20-hour photoperiod was more conducive to vigorous growth and a second flush than are natural summer daylengths at Chicago but did not result in continuous terminal growth, and all buds became dormant on 20-hour photoperiod. A 9-hour photoperiod did not allow vigorous expansion, and no second flush occurred.

The age attained by individual leaves and the onset of abscission under natural conditions may be related to naturally decreasing daylength. Leaves expanding and maturing on a constant 20-hour photoperiod persisted much longer than did those expanding and maturing on a 9-hour photoperiod, but both constant photoperiods resulted in retarding abscission compared with leaves expanding, maturing, and abscising on natural daylengths.

Experiment III. Bud-break and leaf abscission, 1943

The objectives were to test effects of the past treatments and behaviors of the different populations upon their activities in 1943. Observations in detail were limited, however, because of full-time commitment to war work.

Procedure.—On March 12, 1943, 294 dormant plants judged to be alive were removed from their different environments of experiment II and were placed together in an unheated greenhouse room (minimum temperatures above freezing) for chilling on natural photoperiod. On March 30 they were placed outdoors and thereafter during April experienced occasional freezing. All remained outdoors until December 6 (the beginning of experiment IV). Since it was expected that the depth of bud rest on March 12, and as modified by subsequent chilling, would be conditioned to a considerable degree by number and time of flushes of growth in 1942 and by length of persistence of green leaves in relation to both number of flushes and photoperiodic treatment, the population was reassorted into three major series of plants which had shown no, one, or two cycles of growth in 1942; these were represented by 45, 159, and 91 plants, respectively. Within each series plants were arranged according to the time at which leaf-fall had occurred in the autumn and winter of 1942–43. Twenty-five plants still bearing markedly green leaves on March 12 were not included; these were all from subseries B20 and C20 (unchilled or medium chilling in 1942 and on 20-hour photoperiod) and had renewed growth very late in 1942. Practically all other plants were leafless but undoubtedly in different depths of rest.

Results.—The chilling treatment provided was not completely adequate to reduce bud inhibition to a minimum, so that renewal of growth was less prompt and vigorous with onset of warm weather than was expected. Many plants did not show bud expansion until June and some not until early July. By July 30, however, all plants which broke dormancy and attained full leaf had reached the latter stage. The variation in delay could be correlated to a considerable degree with previous treatment and behavior. Thus, on June 29, approximately 20% of the plants producing one or two flushes in 1942 and which had been on either 9-hour or natural photoperiod had

fully expanded their leaves, whereas none of those from the 20-hour 1942 subseries had done so. At the same time half the plants which made two flushes in 1942 were still dormant, whereas 85% of those of one flush had partially expanded. Thus, the persistence of green leaves to a later date and the second flush in the subseries on 20-hour photoperiod in 1942 were correlated with a delay in renewal of growth in 1943 (and indicated differences in depths of bud rest which persisted after the chilling treatment). In this connection, it is of interest that many buds first expanded and grew rapidly during the first very hot weather of the year, June 20–27, suggesting an effect of these natural high temperatures in completely breaking dormancy; this can be compared with the well-known effects of the "warm bath" method in forcing dormant branches (**33**).

All plants of one or two flushes in 1942 broke dormancy in 1943. All those of two flushes in 1942 survived to full leaf and abscission in 1943, and 36% made two flushes. Eight per cent of those of one flush in 1942 succumbed to a "wilt" of unknown etiology before or just after attaining full leaf in 1943, but the remainder survived to abscission, and 31% of them made a second flush.

The behavior of plants in 1943 which did not grow at all in 1942 is of interest. Of the forty-five judged to be still alive on March 12, 1943, thirty-eight had been on 9-hour or natural photoperiod in 1942; twenty-two failed to grow in 1943 and were eventually discarded as dead while dormant. The other twenty-three grew weakly; twenty-one had attained various degrees of expansion by June 29, some even beginning before March 12; *this renewal of growth thus occurred after all buds on these plants had been dormant for 15–18 months or more.* Nine of these plants reached full leaf, but six of them died from "wilt" very soon afterward. Most of the other fourteen partially expanded their foliage leaves, but all succumbed to "wilt" or died from other causes before reaching full leaf. This high mortality was undoubtedly correlated with their greater susceptibility resulting from their lack of vigor induced by past history. The three plants surviving to abscission in 1943 were chilled in the winter and observed for the following year, during which they showed essentially "normal" behavior.

It was impossible to make critical observations during the period of abscission. The plants were left outdoors on natural daylength during this time, and casual observations indicated a time sequence in loss of green color and in abscission in middle and late October only slightly delayed beyond the usual dates for the species, in spite of the late renewal of growth in most plants. These observations again suggested that the decreasing natural daylength in October is a most important factor in affecting the time of abscission. Minimum thermometers among the plants showed the first subfreezing air temperature on November 4, but there were no continued subfreezing temperatures until the second week in November. By this time abscission was complete.

CONCLUSIONS.—Differences in depths of bud rest, as revealed by performance after insufficient chilling to break rest completely, were related to past history in the preceding growing season. Those conditions during that season (especially a 20-hour photoperiod) which favored two flushes of growth, or the persistence of green leaves to a late date, resulted in greater depths of bud rest persisting after inadequate chilling.

Natural high temperatures in summer

may be stimuli to completion of bud rest and foliation of plants delayed in bud-break in areas of mild winter climate.

Some sugar maples can survive for 18 months or more in a completely dormant condition when unchilled and will then resume growth when chilled.

Experiment IV. Bud-break and leaf abscission, 1944

The objectives were to determine (*a*) more critically the effects of differential exposure to cold on the time and rate of renewed growth of dormant buds when placed in warm temperatures (and the indicated reduction in depths of bud rest), (*b*) the ages attained by leaves expanding at different times during the year as an aftereffect of such differential chilling, and (*c*) the degree to which decreasing natural photoperiod could minimize the effects of such differences in leaf age in inducing leaf senescence and abscission.

Procedure.—On December 6, 1943, the surviving plants were reassorted into ten series, one of fourteen plants (series 1), the other nine of twenty-five each. These series were made as comparable as possible in inclusion of plants of differential past behavior and treatment, and it is thought that the results obtained from the various series can be compared directly without regard to the nonuniformity in past history among the plants of each series. All plants had been treated identically since March 12, 1943, and had been outdoors since March 30. On December 6 all had received considerable exposure to subfreezing temperatures in the preceding month, although the minimum air temperature recorded by thermometers set among them had not been below 25° F. and on most dates not below 28° F. It had been below 40° F., however, on all nights since November 1. Hence, there had been enough exposure to temperatures effective in meeting the chilling requirement for partial completion of the rest period.

These ten series, numbered consecutively, remained outside until they were brought in on the dates shown in table 4. In order (except for series 2 and 3) each series was exposed to the outdoor winter temperatures for 6–10 days longer than the preceding series. Series 1 did not experience minimum air temperatures below 25° F. During the night of December 12, after having been above freezing the preceding week, the air temperature dropped to 8° F. and had only risen to 18° F. when series 2 was brought in. Thus series 2 experienced several hours of much more intense cold than did series 1. Series 2 and 3 differed by 23 hours of such exposure but, as brought out later, in relation to this slight difference, diverged more significantly in their responses than did any two other consecutive series; the air temperature dur-

TABLE 4

MEAN VALUES OF DATA FOR EXPERIMENT IV

Series	Date brought in	No. days to bud-break*	Mean date of bud-break	Mean date of abscis-sion	No. days, bud-break to abscis-sion*
1....	Dec. 6	109.5	Mar. 25	Oct. 22	211.0
2....	Dec. 13†	84.3	Mar. 6	Oct. 24	231.8
3....	Dec. 14‡	69.4	Feb. 21	Oct. 20	242.0
4....	Dec. 20	70.3	Feb. 28	Oct. 24	239.0
5....	Dec. 27	58.6	Feb. 24	Oct. 28	247.2
6....	Jan. 4	56.5	Feb. 29	Oct. 24	238.0
7....	Jan. 14	49.7	Mar. 4	Oct. 24	234.0
8....	Jan. 24	41.8	Mar. 6	Oct. 30	238.4
9....	Feb. 5	45.2	Mar. 21	Nov. 2	226.0
10....	Feb. 15	39.4	Mar. 25	Oct. 29	217.5

* Based on averages of individual plants, not on intervals between mean dates.

† 4:30 P.M.

‡ 3:30 P.M.

ing this 23-hour period remained between 10° and 18° F. All the other series (4–10) experienced longer periods of severe chilling, especially between December 14 and January 14; the minimum air temperature recorded among the plants outdoors during this month was 4° F., and the minimum was below freezing on all but two nights. The daytime maxima from December 14 to January 14 ranged from 11° to 44° F., mostly between 20° and 40° F. After January 14 the minimum air temperature was above 26° F. each night until February 11, and on many nights was above freezing. During this latter period daytime maxima were always above freezing and ranged as high as 62° F. Series 9 was exposed to very little more freezing temperature than was series 8. Series 10 experienced severe subfreezing temperatures (minima to 4° and 7° F.) during the 2 days just before it was brought in.

As the series were brought inside, they were placed on an open bench on natural photoperiod in a greenhouse thermostatically controlled for a temperature range of 59°–77° F. (usual maximum was 72° F.) during December, January, and February. The maximum ranged slightly higher in March and April (up to 84° F.). These temperatures were high enough to allow expansion of buds of sugar maple upon completion of their rest and relatively rapid expansion of leaves but are probably not low enough to be effective in satisfying the chilling requirement.

BUD-BREAK.—From February 5 to April 15 plants were observed individually at intervals of 2–7 days to determine the numbers and percentages of plants in each series (*a*) still dormant, (*b*) showing bud expansion but not in full leaf, (*c*) with fully expanded leaves, and (*d*) apparently dead. Thereafter, until June 15 when all plants had reached the full-leaf stage (or obviously had failed to grow), such observations were recorded at intervals of 7–15 days. Twenty-nine of the 239 plants failed to renew growth in the greenhouse and were obviously dead in August. These were distributed as 1, 0, 0, 0, 4, 2, 1, 2, 8, and 11 plants in series 1 through 10, respectively. This mortality, especially in series 9 and 10, and probably in series 5–8, was possibly due to winter killing resulting from prolonged winter exposure with roots in small pots or, especially in series 9 and 10, as a consequence of reduction in resistance to winter injury upon completion of rest. It is possible that the one plant failing to grow in series 1 had been insufficiently chilled. These twenty-nine plants have been eliminated in analyzing the data on bud-break, leaf expansion, and abscission. A few plants which renewed growth died from "wilt" or other causes at various times before leaf-fall.

The length of time required for terminal buds to show obvious swelling after the plants were brought into the greenhouse ranged from 24 to 167 days (table 5). Such expansion was judged by easily visible elongation of scales, and it is not implied that no growth occurred during this period. The similarity in mean length of this interval in series 8–10 (table 4; fig. 3) suggests that bud rest was essentially at a minimum at the time these series were brought in, and it may be assumed that it requires about 35–40 days of exposure to temperatures warm enough for growth before expansion becomes visible externally from dormant buds of this species whose rest had been approximately completed. The assumption is in line with the behavior of the species in spring in the Chicago region. It required 30–50 days in tem-

peratures ranging from 40° to 70° F. for plants of series A (maximum chilling) in experiment II to show bud swelling. DEUBER and BOWEN (15) reported that 2 months elapsed before dormant sugar maples were partly leafed out after a 5-day exposure to ethylene chlorhydrin. If this period (35–40 days) is subtracted from the lengths of the intervals (shown in table 4) between bringing-in and bud-break for series 1–7, the effectiveness of continued exposure to cold on completion of rest is even more obvious; and especially the effect of the 23-hour period of severe subfreezing temperatures in reducing bud inhibition in series 3 as compared with series 2 (fig. 3).

The mean length of the interval between bringing-in and bud-break was shorter, in general, for each successive series, but the greatest change in average degree of bud rest per additional day of cold exposure (fig. 3) is obviously between series 2 and 3, and then between series 1 and 2. If these data are converted to approximate mean dates of initial bud swelling for each series (table 4), it shows that all series had reached the bud-swelling stage 5–6 weeks (or more) sooner than they do in the forests near Chicago. Only two plants (one each in series 1 and 2) of those which eventually grew had failed to show bud swelling by May 1. It may be assumed that rest in this species in this region may be essentially complete in February.

The more nearly uniform response in time of bud-break within each of series 5, 6, and 7 (table 5) suggests near-completion of rest for most plants when these series were brought in. Greater diversity in the earlier series was probably related to difference in depths of rest remaining after insufficient chilling, while the spread in the later series may have been related to delay in some plants resulting from winter injury occurring after rest was completed.

Thus, completion of bud rest as a consequence of chilling is a gradual process in this species, as in most temperate-zone deciduous woody plants, and presumably requires several hundred hours of exposure to cool temperature. But the effect of the 23-hour exposure to severe subfreezing temperature in series 3 in

TABLE 5

PERCENTAGE FREQUENCY DISTRIBUTION WITHIN SERIES, FOR 10-DAY INTERVALS, OF TIME REQUIRED FROM BRINGING-IN TO BUD-BREAK. EXPERIMENT IV

SERIES	NO. PLANTS AS BASIS	INTERVALS (DAYS)													
		24–30	31–40	41–50	51–60	61–70	71–80	81–90	91–100	101–110	111–120	121–130	131–140	151–160	161–167
1	12					8			17	42*	8		17		8
2	25					28	28	16*	12	12					4
3	24			4	12½	37½*	29	12½	4						
4	25				28	20*	28	16	8						
5	21			14	52*	33									
6	23		4	30	35*	13	17								
7	24		8	67*	12½	8				4					
8	23	4	43½	43½*		4		4							
9	17	24	6	47*	6	12	6								
10	13	.....	77*	17	6										

* Mean (see table 4) falls in this interval.

accelerating the completion of rest by nearly 2 weeks (fig. 3; table 4) in comparison with series 2 is the most interesting result, since it is widely accepted that freezing temperatures in such plants are no more effective in this process than are those ranging approximately between 32° and 45° F. This result is also of interest in terms of speculation as to chemical or physiological changes which may have occurred in the buds as a consequence of such exposure.

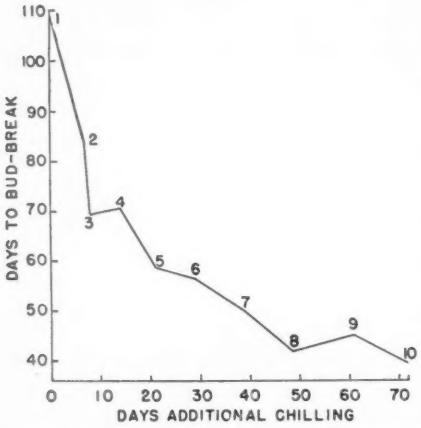


FIG. 3.—Acceleration of bud-break in sugar maple with additional winter exposure. Number of days of additional chilling plotted against mean number of days required for visible bud swelling within various series (1–10) consecutively brought into a warm greenhouse from outdoors at intervals starting on December 6 (series 1). Note especially pronounced effect of 23 hours of additional exposure for series 3, compared with series 2.

GROWTH.—Practically all plants showed fairly rapid expansion of buds and leaves once bud-break had occurred, in spite of differences in chilling. All had received some chilling, however, in comparison with some of the plants in experiment II which grew weakly when unchilled. There were no consistent or marked differences among series, the mean lengths of the intervals between dates of bud-break and dates for completion of leaf expansion and terminal extension ranging from 22 to 32 days; most series fell between 25 and 29 days. Only ten plants of those which grew at all, from series 1, 2, 7, 9, and 10, were not in full leaf by May 1. Practically all plants attained a full-leaf status from 1 to 3 months earlier in the year than under field conditions in the local area.

Terminal growth also stopped proportionately earlier in the year, and only a few plants showed a second flush. This latter fact may possibly be attributed to the increase in the potbound condition with increase in age and size of plants, but it is not thought that this was sufficiently serious to have overcome any strong stimulus toward prolongation of growth or toward a second flush which might have resulted from the increasing natural photoperiod in the longest days in May and June. It may be recalled that sizable percentages of the populations on natural photoperiod showed second flushes in each of the three preceding years in July, August, and September.

LEAF SENESCENCE AND ABSCISSION.—The plants remained in the warm greenhouse in the autumn. Loss of green color and abscission were observed at intervals of 2–3 days as in 1941 and 1942, except that, when all leaves on a plant were almost completely brown, they were subjected to uniform mechanical disturbance at each period of observation to simulate more nearly outdoor conditions for pressures from wind movement, rain, etc. When such pressures had resulted in detachment of all leaves from an individual plant, the date was recorded as its abscission date. Lengths of the intervals between dates recorded as "brown" and "abscission" were mostly less than 1 week with a few as long as 3

weeks and a few as short as 2–3 days. There was no relationship to series in such duration.

Mean dates of abscission for each series were calculated from the data on individuals and showed a sequence (table 4) slightly related to age of leaf as conditioned by the sequence of bud-break and leaf maturation (table 4). Series 3 showed earliest leaf-fall, series 8, 9, and 10 the latest. But it is obvious that the time of senescence and abscission was much less related to actual age of leaf than to an induction by external influences. Mean dates for abscission are very similar (slightly delayed) to those of the species in the region. Since temperatures in the greenhouse had been maintained above 65° F., it is concluded that the decreasing natural photoperiod in late September and October, even under warm conditions, is a powerful stimulus to senescence and leaf-fall in this species, as was earlier concluded from the data of experiment II. This is all the more striking when it is recalled that these leaves were already 1–3 months older than they ever become under natural conditions in the region but had not shown the onset of senescence earlier. For the total of 195 plants under observation the last leaves from individual plants all fell between October 6 and November 13, and for over 75% of the individuals between October 13 and November 1.

The variation in age attained by leaves of individual plants, as measured by the intervals between bud-break and abscission dates, is shown in the percentage frequency distributions in table 6. Mean leaf ages for series are shown in table 4. Lowest ages were found chiefly in series 1 and 2 and represent plants which were delayed most markedly in bud-break, probably because of insufficient chilling. Maximum ages were found chiefly in series 2–6 and represent plants which had been sufficiently chilled when brought in to show bud-break soon after, and hence could attain greater leaf age than those, also soon expanding, which were brought in later, since abscission occurred more nearly simultaneously. How much greater the age leaves might have attained if the plants

TABLE 6

PERCENTAGE FREQUENCY DISTRIBUTION WITHIN SERIES, FOR 10-DAY INTERVALS, OF LEAF AGE (BUD-BREAK TO ABSCISSION). EXPERIMENT IV

Series	No. plants as basis	Intervals (days)												
		157–160	161–170	171–180	181–190	191–200	201–210	211–220	221–230	231–240	241–250	251–260	261–270	271–272
1	12		8		8	8	8	42*	8	8	8			
2	24	4				4	4	8	21	21*	25	4	4	4
3	22								9	36	45*	5	5	
4	24							8	8	42*	29	12½		
5	20								5	20	40*	25	10	
6	22							9	14	41*	18	18		
7	22					5			23	55*	14	5		
8	21								19	38*	43			
9	12							33	33*	25	8			
10	13					8		54*	23	15				

* Mean (see table 4) falls in this interval.

were maintained on long photoperiods (as in experiments I and II) or how much sooner they might have been induced to abscise by an earlier gradual decrease in photoperiod simulating conditions in September and October is unknown. That abscission can be induced at a much earlier age by the decreasing natural daylength is shown in experiment V.

CONCLUSIONS.—Dormant buds of sugar maple require several hundred hours of chilling for satisfactory completion of their rest. The process is somewhat gradual in relation to added increments of chilling. In the Chicago area rest in native individuals may be presumed to be complete by February. At one stage in rest differential exposure to temperatures below freezing may produce quite marked effects on completion of rest; 23 hours of additional exposure to temperatures of 10°–18° F., after considerable previous chilling both below and above freezing, accelerated bud-break by approximately 2 weeks, even though rest was still not completed. This is in contrast to general findings and assumptions that temperatures below freezing are not so effective (or no more so) as those of 32°–45° F.

Leaves expanding on plants from February through May under warm greenhouse and natural photoperiodic conditions persist until October and may reach ages up to 3 months older than do those in the normal outdoor growth cycle. Yet such leaves inside are all abscised at approximately the same time in October and early November, irrespective of their chronological age. This indicates that the decreasing natural photoperiod preceding normal leaf-fall is a powerful stimulus to senescence and abscission.

EXPERIMENT V. ABSCISSION, 1947

The objective was to observe any apparent effect of decreasing natural daylength in 1947 in inducing abscission of leaves of much younger chronological age than those tested in experiment IV. At the conclusion of the observations in 1944 the plants were chilled outdoors for completion of rest, then maintained under greenhouse conditions in 1945, again chilled in the winter of 1945–46, and left under greenhouse conditions for the growing season of 1946. They were used in an experiment—to be reported elsewhere—in the late summer and autumn of 1946 and were then placed in a cool greenhouse during the following winter but not subjected to prolonged chilling. As planned, the amount of chilling received was inadequate to break their rest completely, so that growth was resumed over an extended period of time in 1947 by the different individuals.

Beginning with the first bud-break on April 22, 1947, bud-break and leaf expansion were recorded at weekly intervals until August 23, when bud-break had occurred in all 147 individuals which survived to later abscission. Approximately one-fourth of the plants had shown bud-break by May 14, one-half by May 27, three-fourths by June 23, and all but 6 of the remaining one-fourth by July 27. From plant to plant leaves in the autumn differed in age by a maximum of 4 months (from April 22 to August 23 for first and last bud-break).

After loss of green color, abscission of leaves in a warm greenhouse in October and November was brought about by slight pressures as in experiment IV, and the date of fall of the last leaf per plant was recorded as its date of abscission. These dates all fell in the period of Oc-

tober 22–November 14. Approximately one-fourth of all plants had lost all leaves by October 31, one-half by November 4, and three-fourths by November 9. The extreme ages of leaves on individual plants, as measured from bud-break to abscission, were 74 and 206 days; these minimum and maximum values are both much lower than those in experiment IV (157 and 272 days) in which most buds and leaves had expanded 1–3 months earlier than the normal period for the species in this region. The percentage frequency distribution of range in maximum leaf age per plant in 10-day intervals is as shown in Table 7.

CONCLUSIONS.—Since these leaves of widely differing ages all abscised over a period of only slightly more than 3 weeks, abscission must have been induced by factors sufficiently strong in their stimulus to overcome almost completely the age-of-leaf effect noted in experiments I and II. Since many of these leaves were much younger than the leaf age attained in the species under outdoor conditions, since the plants were in a warm greenhouse, since there were no known injurious external agencies which might have induced abscission, and since they were abscised at approximately the same time of year as the much older leaves in experiment IV, the conclusion drawn from experiment IV seems amply confirmed by these data—that the decreasing natural photoperiod in September and October operates to promote senescence and abscission in this species, in this case sufficiently so as to induce abscission of some leaves only 2–3 months old.

Discussion

Ecologically, the results of these experiments may be considered in relation to the seasonal cycle in sugar maple and to its geographical distribution, as well as in their specific implications for photoperiodism and winter chilling. Physiologically, they may be considered in relation to their bearing on the internal mechanisms leading to leaf abscission, bud dormancy, and bud-break, and especially to the photoperiodic control of these mechanisms.

TABLE 7

Maximum leaf age (days)	% of total population
71– 80	1
81– 90	1
91–100	3
101–110	3
111–120	2
121–130	12
131–140	8
141–150	12
151–160	14
161–170	16
171–180	14
181–190	10
191–200	3
201–210	1

It seems plausible to suggest that, for the population investigated, the naturally decreasing photoperiod at this latitude is the primary stimulus to leaf senescence (as judged by loss of green color) and abscission in September, October, and November. The effect of this stimulus is conditioned to a slight extent by the chronological age of leaves, younger leaves being more slowly affected. Most leaves expanding from as early as February to as late as August became senescent and were abscised in October and November if the plants had been maintained on natural daylength, either indoors or out. The only exceptions were in subseries BN and CN of experiment II in which leaves expanding in August or later persisted into December. These leaves expanded from buds

which broke dormancy with limited or no chilling; those persisting through December (fig. 2) were on the few plants within subseries CN which showed a second flush after August.

It may be argued that under natural ecological conditions other factors, such as diurnal temperature fluctuations, cool night temperatures (not freezing), etc., may be equally (or more) important in inducing senescence and leaf-fall. But these experiments demonstrate that in this species a physiological mechanism exists which can result in these visible phenomena, apparently in response to decreasing photoperiod, even in the absence of marked diurnal temperature fluctuations or of cool night temperatures and at approximately the same time as in ecologically natural situations. Since the species outdoors at this latitude shows consistent chronological behavior in leaf-fall from one year to another, in spite of temperature differences in successive years, it seems plausible to assume that this photoperiodic mechanism usually does basically determine the natural time of occurrence of this event. This, however, does not imply an absolute, single-factor control.

The physiology of this mechanism is obscure. It may involve loss of green color as one step, but this loss may be a side effect of the aging process related to photoperiod and may not in itself directly lead to abscission. The writer has not experimentally determined whether leaf-fall in this species can be chemically induced before loss of green color, but under field conditions not many of its leaves are shed with much green area still visible, nor were they in these experiments. These facts are of interest in connection with the hypothesis of GAWADI and AVERY (**21**), since loss of green color could be related to or parallel with both the cessation of synthesis of materials inhibiting abscission, such as auxin, and the accumulation of materials, such as ethylene, which induce it. The relation of the formation and/or translocation of either class of substance to photoperiodic control of the type suggested here is not clear. The effect of decreasing natural photoperiods in accelerating abscission (as compared with the effects of either long or short constant ones) may involve the shifting of gradients rather than absolute levels of inhibitors and/or activators of abscission. The change in balance between activators and inhibitors need not necessarily be directly related to any simple absolute ratio of the length of light to the length of dark period, such as seems to have been implied by WAREING (**43**) to account for the photoperiodic mechanism controlling growth in *Pinus silvestris*. Experiments on additional species showing photoperiodically induced abscission should be clarifying, however, especially if photoperiodic cycles over a wide range of light-dark ratios were used, and including cycles more than 24 hours long, cycles with dark-period interruption, and cycles with continuously shifting light-dark ratios to simulate natural changes in daylength. Such an approach, combined with the recent one of SHOJI *et al.* (**36**) on auxin gradients in relation to abscission and taking into consideration the hypothesis of GAWADI and AVERY (**21**), might considerably advance our knowledge of the physiology of natural abscission in aging leaves in which the leaf has not been mechanically injured or subjected to abnormal chemical treatment.

The onset of terminal bud rest is not readily related to natural photoperiodic conditions. In these experiments terminal growth stopped and winter buds were differentiated on photoperiods as

short as 9 hours, as long as 20 hours, and while natural photoperiods were increasing or decreasing—from March to the long days of June and from June to November. Development of terminal bud rest was thus not prevented by any of the photoperiodic conditions employed, although it was delayed to some extent by the 20-hour photoperiod in experiment II which stimulated a second flush before buds went into rest. Although growing points were not examined during the second flush, the extent of this flush was presumably not so closely related to the elongation and maturation of a morphologically predetermined number of leaf and scale initials as was that of the first flush; probably the entire complement of leaf and scale primordia had not yet been differentiated in the buds before the second flush began. Yet the second flush was soon terminated, either on 20-hour or on natural photoperiod of varying lengths. The stimulus to growth resulting from long days evidently either cannot be maintained or is soon outweighed in this species by other factors leading to terminal bud inhibition and maturation. A constant 9-hour photoperiod did, however, completely prevent any plants from making a second flush. These facts, together with the delay of bud-break in plants on 9-hour photoperiod after some or no chilling in comparison with plants on natural and 20-hour photoperiods, suggest that the development of terminal bud rest in this species may be related to a photoperiodic mechanism. Experiments with this species on continuous light might answer this problem. The present results indicate, however, that the development of terminal bud rest is a complex, multiconditioned process and that in this species a precise photoperiodic mechanism is rarely, if ever, alone decisive in leading to such rest.

The same conclusion applies to the breaking of rest. Undoubtedly this process is naturally accomplished gradually in sugar-maple buds in deep rest as a consequence of adequate chilling. With no or inadequate chilling for completion of rest, an exposure to 20-hour photoperiod or natural day (in late spring and summer) apparently allowed or induced earlier and higher percentages of bud-break than did a 9-hour photoperiod. In areas of mild winter climate with inadequate chilling for some woody species it is conceivable that the long days of summer do influence the extent and time of their bud-break in that growing season. No speculation is offered as to the possible physiology of this effect in leafless twigs, although there certainly are enough pigments in bud scales and stem chlorenchyma to allow photochemical effects. Other natural stimuli, such as heat, or aging alone may result in overcoming inhibition in some plants.

Ecologically, the experiments on chilling in relation to completion of rest indicated that in general the population tested had a high chilling requirement adequately satisfied by the usual winter weather in the Chicago region. The importance of this requirement as a distributional factor in the native range is suggested, but native populations from other parts of the range must be tested to show whether the amount of chilling required varies geographically. Natural selection may have resulted in geographical differentiation in regard to this factor, and trees from southern parts of the range might show lower requirements. The variation in chilling need within the tested local population suggests that such diversity on a geographic basis might be found. It is obvious, however,

that areas of too mild a winter climate would preclude the successful cultivation of any sugar maples having chilling requirements similar to those of most of the individuals tested; presumably this requirement may be a limiting factor in the horticultural distribution of the species, as it is for so many woody plants native to cold temperate regions (**10, 24**).

In these chilling experiments, as in those elsewhere on other species, the internal factors affected by chilling, which control bud inhibition, resist definition. The most significant and provocative result was the pronounced effect of the additional 23 hours of exposure to subfreezing temperatures in accelerating bud-break in series 3 of experiment IV. This is in contrast to the widespread opinion that subfreezing temperatures are not so effective (or no more so) in breaking rest as are temperatures slightly above freezing. No speculations are offered as to the physiology of this response, but a re-examination of the effects of freezing might be useful for investigators on the physiology of rest in buds of such woody plants.

Summary

1. A seedling population of sugar maple (*Acer saccharum* Marsh.), derived from two native seed trees in southeastern Wisconsin, was grown for 7 years (1941–1947) in pots in the University of Chicago greenhouses and gardens. The experimental variables were mainly length of photoperiod and amount of winter chilling, studied particularly in relation to leaf senescence and abscission, onset and completion of bud rest, budbreak, and amount of terminal growth.

2. It was concluded that in this species the loss of green color and leaf abscission under natural conditions are basically conditioned by the naturally decreasing autumnal photoperiod. In the various years and experiments leaves expanding from as early as February to as late as August were mostly abscised in October and November if the plants were maintained on natural photoperiod, either indoors or out. Chronological age of leaves had a limited relation to time of senescence and abscission, youngest leaves persisting a few days longer.

Constant photoperiods of 16, 12, and 8 hours, imposed in the greenhouse on first-year seedlings on September 10, resulted in delayed senescence and abscission (up to 5 months for youngest leaves on 16-hour photoperiod) compared with plants outside on natural photoperiod. There was considerable variation in time of these events within any one treatment, but in general the amount of delay was positively related to length of constant photoperiod. A change from 16- to 8-hour photoperiod in December accelerated senescence and abscission as compared with plants remaining on 16-hour photoperiod.

In second-year plants leaves of similar chronological age persisted longer when they had expanded and had been maintained inside on 20-hour than on 9-hour light periods; but those on natural photoperiod lost their green color and were abscised in October and November, generally earlier than those on either constant photoperiod.

In all experiments loss of green color always preceded abscission.

These results all suggest that the natural photoperiodic mechanism leading to senescence and abscission may involve shifting gradients of abscission inhibitors and/or activators conditioned in a complex fashion by changing light-dark ratios in the photoperiodic cycle. Thus, leaves were eventually abscised on any of the photoperiodic treatments em-

ployed, but their chronological age had much more influence on the length of their persistence when they were on constant than when on natural photoperiods. Presumably decreasing photoperiods critically condition the aging process leading to abscission.

3. Completion of bud rest and bud-break were studied in relation to winter chilling, as modified by the effects of past history and current photoperiod. In general, the population tested apparently required several hundred hours of chilling to complete bud rest and to allow bud-break at the normal time. This need varied from plant to plant both genetically and in relation to depths of rest as conditioned by past history. Some plants eventually grew without chilling, but some unchilled plants remained dormant for 15–18 months and then grew after chilling.

After no or inadequate winter chilling in 1941–1942, exposure to 20-hour photoperiod after April 28 or to natural long days of spring and summer induced bud-break in higher percentages of plants, and more vigorously, than did a 9-hour photoperiod. Only 35% of unchilled plants eventually grew when on 9-hour photoperiod; but 64% and 54% had grown by September 15 on natural and 20-hour photoperiods, respectively. Thus, in some plants, but not all, inadequate or no chilling may be partially compensated for by long photoperiods. A 16-hour photoperiod imposed in September, however, did not stimulate any newly formed buds to grow.

In some, but not all, cases plants inadequately chilled or unchilled were more delayed in bud-break if they had continued growth later, or had green leaves persisting longer, in the preceding growing season.

After chilling outdoors until December 6, populations brought into a warm greenhouse at intervals until February 15 showed proportionately earlier bud-break the longer the exposure, until the last week in January when rest was apparently nearly completed. The effect of additional exposure in accelerating bud-break was more pronounced in December than in January. The most interesting effect in such acceleration resulted from 23 hours of additional exposure to temperatures of 10°–18° F. on December 13–14. Mean date of bud-break for plants receiving this additional exposure was advanced 2 weeks over those brought in the day before.

There was some evidence that winter injury was more likely to occur after rest was completed.

Chilling requirement is undoubtedly a factor in the horticultural distribution of this species. Its importance in natural distribution can only be suggested until other native populations are tested for possible geographic variation in chilling needs.

4. Duration and extent of terminal growth were related to photoperiod. Individual leaves expanding on 20-hour or long natural photoperiods were larger than those expanding on 9-hour photoperiod. Internodal extension was not measured. Duration of terminal growth was similar for first flushes, irrespective of photoperiod, but, in 1942, 70% and 23% of plants on 20-hour and natural photoperiods, respectively, made a second flush, whereas none did on 9-hour photoperiod. Long days are temporarily stimuli to extension for buds not in deep rest.

5. Terminal buds became dormant on photoperiods ranging from the natural short days of March and November to the longest ones of June, and on 9- and 20-hour constant photoperiods. The

development of bud rest under natural conditions is thus not decisively controlled by photoperiod, even though some results suggest that daylength may contribute secondarily—as, for example, in the lack of a second flush of growth in any plants on 9-hour photoperiod.

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BROWN-SPOT DISEASE OF CELERY[1]

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Introduction

In September, 1943, several bunches of Pascal celery (*Apium graveolens* L.) affected with a disease not previously seen on the Chicago market were received from the Fresh Food Products Inspection Service of the United States Department of Agriculture in Denver, Colorado. The accompanying report stated that the disease was evident on both Pascal and Golden Self-Blanching varieties grown in the vicinity of Denver. Incidence of infection ranged from 5% to 85% in celery fields of the various growers.

Celery affected with this disease had been received from the same Colorado district during the years 1944–1949 inclusive. A preliminary report on the disease was published in 1948 (**15**). This paper is the result of further studies concerning the pathogenicity, physiological characteristics, morphology, and taxonomy of the causal organism.

Observations

Symptoms

The brown-spot disease is characterized by irregular tan to brown, shallow lesions, 2–15 mm. in diameter, occurring on the inside and outside of leaf stalks, on petiolules, and on leaflets (fig. 1*A–E*). Although the outer leaf stalks may show abundant infection, the more central stalks within the bunch are rarely infected. Lesions are often so numerous that they coalesce to form areas extending several centimeters along the stalk

[1] Investigations conducted by the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, U.S. Department of Agriculture in co-operation with the Department of Botany at the University of Chicago.

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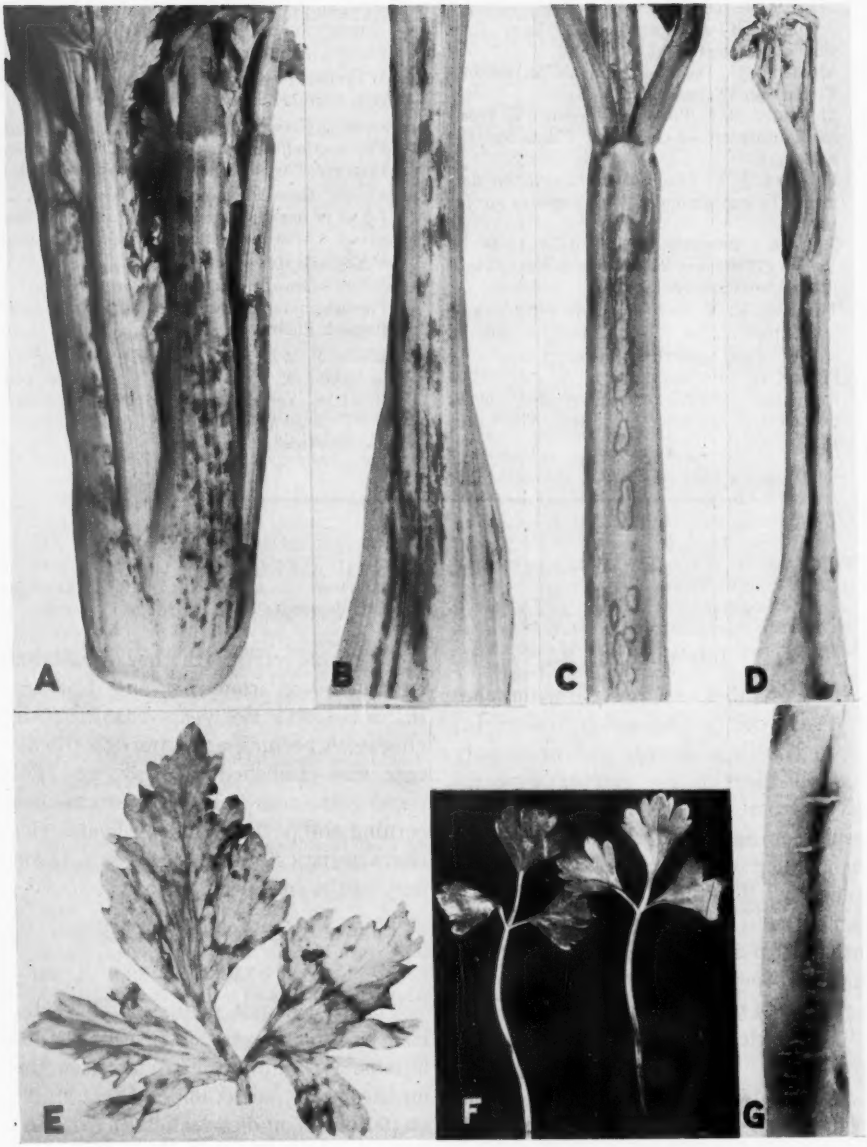

FIG. 1.—*A*, Pascal celery affected with brown-spot disease. *B*, infections on outer stalk of bunch. *C*, coalesced infections on inner curve of stalk. *D*, distortion of infected stalk. *E*, leaflet infections. *F*, girdling of seedling petioles following infection. *G*, cracking following petiole infection.

(fig. 1*C*). Infection may also result in a curling or distortion of mature stalks (fig. 1*D*). Small cracks at right angles to the vascular bundles are often apparent in diseased areas (fig. 1*G*). Lesions on petiolules are yellowish-brown to brown and smaller than those on main stalks. Infections on leaflets are most often apparent as yellowish-brown areas in the axils and in leaflet notches but are not sharply defined (fig. 1*E*).

Microtome sections of affected stalks and petiolules show that the disease is confined primarily to the epidermal cells, although occasionally one or two layers of subepidermal cells may be invaded.

An examination of diseased tissue that has remained in an atmosphere of high relative humidity for 6 or more hours reveals a fine surface growth of white mycelium that bears numerous spores at the end of short conidiophores (fig. 2*A*). These spores collect into mucilaginous balls that disperse immediately on contact with water (fig. 2*B*), a characteristic shown by members of the genus *Cephalosporium*. During these studies many isolations have been made from lesions on stalks, petiolules, and leaves, and *Cephalosporium* sp. has been repeatedly recovered in pure culture. The literature reveals no reference to the occurrence of this organism on celery or related plants.[4]

At various times attempts were made to isolate *Cephalosporium* from celery seed from fields that had shown infection in successive years; none was successful. Since *Cephalosporium* is well known as a soil inhabitant, it seemed desirable to attempt to isolate the organism from soil of infected celery fields. All such attempts were unsuccessful; but this does not preclude the possibility that the organism is present in the plant bed soil or in celery fields.

[4] After this paper was in press the isolation of *Cephalosporium sp.* from celery in New York was reported by SEGAL (SEGAL, RALPH. Brown spot disease of celery found in New York. U.S. Dept. Agr. Pl. Dis. Reptr. **35**: 164. 1951).

PATHOGENICITY

Inoculation of Pascal celery obtained on the local market was made by flooding the concave surfaces of stalks with a conidial suspension of a 14-day-old culture of *Cephalosporium*. Inoculated stalks were held in moist chambers at room temperature (75°–78° F.), and infections were evident 8–16 days later. Re-isolations of the fungus in pure culture were readily made from diseased tissues.

In greenhouse inoculations six potted, 1-year-old Pascal celery plants were sprayed with a conidial suspension of a pure culture of *Cephalosporium* isolated from infected Colorado celery. After inoculation pots were covered with bell jars for 8 hours; the jars were then removed and the pots placed on the greenhouse bench. After 18 days irregular, yellowish-brown areas were visible on leaf surfaces and in leaf axils. Such material, when cultured, yielded pure cultures of *Cephalosporium*. Further experiments were carried on with 1-year-old plants of Pascal and Golden Self-Blanching celery. Some of the plants were injured either by scratching stalks and leaves with needles or by rubbing them with fine carborundum powder on moist cotton. Inoculations were made by spraying the plants with a conidial suspension of the fungus. Infection of stalks, petiolules, and leaves of injured plants was observed 18 days after inoculation; on noninjured plants, not until 25 days after inoculation. Leaf and petiolule infection was characterized by yellowish-brown areas, 1–3 mm. in diameter, scattered over their surfaces (fig. 1*E*). Infected stalks frequently showed reddening of vascular bundles and cracking that occurred at right angles to the bundles (fig. 1*G*). As indi-

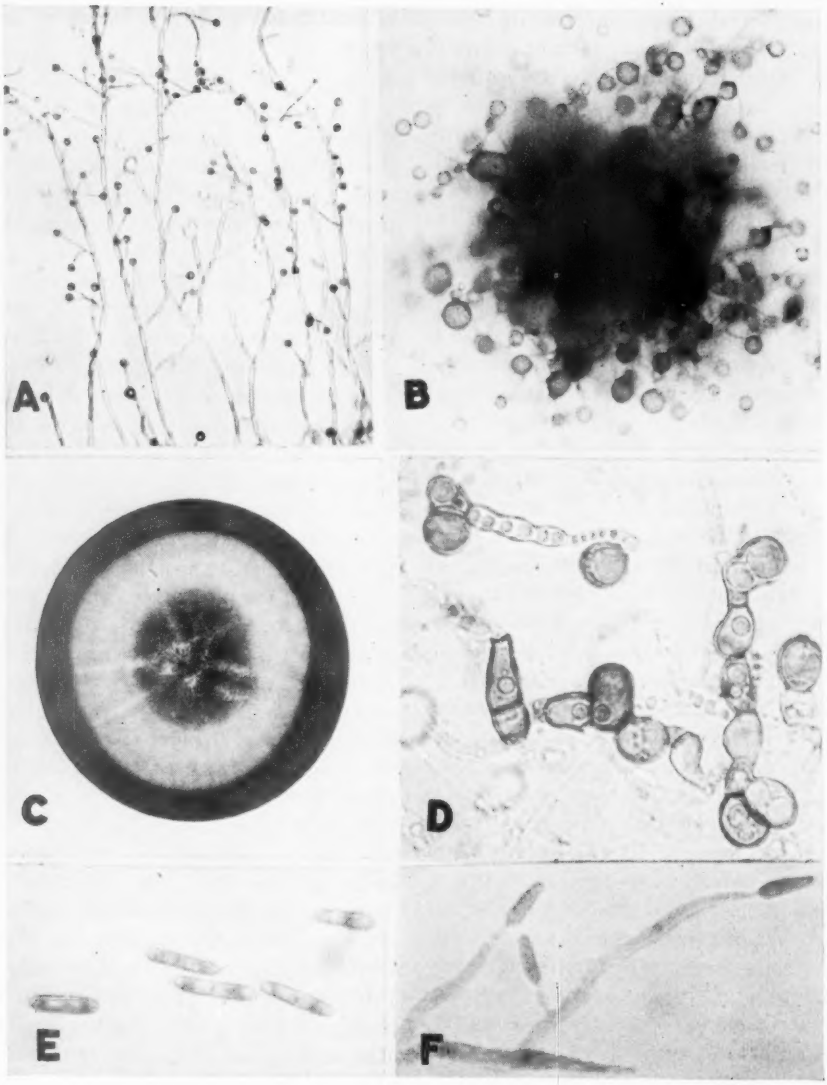

FIG. 2.—*A*, spore heads borne on ends of short conidiophores. *B*, mucous heads borne on aerial conidiophores in celery-dextrose agar. *C*, 21-day-old culture of *Cephalosporium* on potato-dextrose agar. *D*, terminal and intercalary chlamydospores. *E*, conidia. *F*, conidiophores.

cated earlier, this condition was observed also on mature stalks naturally affected with the disease.

Studies were made of the susceptibility of seedlings to the disease. Seed obtained from a field of celery in which the brown-spot disease had not been observed for 3 successive years was planted in each of three 8-inch pots. When the plants were 5 inches tall, one pot of seedlings was inoculated by spraying with a spore suspension of *Cephalosporium* originally isolated from affected Colorado celery. A second pot of seedlings was sprayed with a spore suspension of *Cephalosporium* that had been re-isolated in a previous experiment. The third pot of seedlings, sprayed with distilled water, served as a control. Following inoculation pots were covered with bell jars for 16 hours; the jars were then removed and the pots placed on the greenhouse bench.

After 30 days, 56% of the seedlings in one inoculated pot and 75% of those in the second pot showed infection. Control plants remained free of the disease. Infections were characterized by yellowish to yellowish-brown, slightly mottled, irregular areas on foliage. The most pronounced symptom, however, was a constriction or girdling at points along petioles where infections had occurred (fig. 1*F*). *Cephalosporium* sp. was readily re-isolated from tissue in the immediate vicinity of the girdled areas as well as from lesions on leaflets.

An experiment was made to note the influence of injuries on the incidence of infection of leaflets and petioles of seedlings. As in previous experiments, they were injured by rubbing them with fine carborundum powder on moist cotton, after which they were sprayed with a conidial suspension of the fungus. Controls consisted of injured and noninjured plants sprayed with distilled water.

After 19 days, 66% of the injured and 29% of the noninjured inoculated plants had become infected; after 30 days the values were 82% and 69%, respectively. Control plants remained free of the disease. Thus, injury of seedlings at the time of inoculation resulted in a higher percentage of infected plants. It is apparent, however, that seedling infection was not wholly dependent upon injury.

The susceptibility of certain other members of the Umbelliferae to the brown-spot disease was tested. Seeds of anise (*Pimpinella anisum* L.), caraway (*Carum carvi* L.), carrot (*Daucus carota* L.), dill (*Anethum graveolens* L.), finnochio (*Foeniculum vulgare* L.), parsley (*Petroselinum hortense* Hoffm.), and parsnip (*Pastinaca sativa* L.) were planted in 6-inch pots. When plants were 5 inches tall, the pots were placed in a glass-inclosed moist chamber and sprayed with an aqueous suspension of spores from a 14-day-old culture of *Cephalosporium*. In addition, one pot of celery seedlings was inoculated with the organism and placed in the moist chamber with the other inoculated plants. One pot of each of the above plants sprayed with distilled water and placed in a separate moist chamber served as a control. After 24 hours the pots were removed from the moist chambers and placed on the greenhouse bench where they were under observation for 50 days. At the end of that period none of the anise, caraway, carrot, dill, finnochio, parsley, or parsnip plants had become infected, but 92% of the celery plants were infected with *Cephalosporium*. Control plants remained free of the disease.

In a repetition of this experiment 84% of the celery seedlings had become infected after 50 days. None of the other members of the Umbelliferae inoculated at the same time were infected. Control

plants remained free of the disease. Thus, of the various members of the Umbelliferae inoculated, only celery proved to be susceptible to infection.

CULTURAL CHARACTERS

On potato-dextrose agar the fungus forms slow-growing, circular, slightly raised, cottony-white colonies. There is an abundant production of conidia in the colonies after 7 days' growth. The surface of the colonies in 21-day-old plate cultures becomes grayish owing to the presence of chlamydospores (fig. 2*C*). Growth in celery-dextrose agar is white and cottony and slightly more rapid than on potato-dextrose agar. Spore production is abundant after 5 days, and there is heavy chlamydospore production after 18 days' growth. Growth in steamed rice is very slow. After 21 days there is scant mycelial growth, but chlamydospore production is heavy. The organism makes rapid growth on cooked celery stalks,

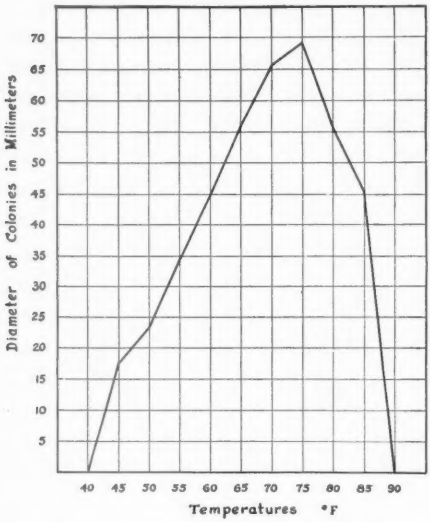

FIG. 3.—Growth of *Cephalosporium* in culture at different temperatures. Average of five isolates.

spore heads bearing conidia being evident 8 days after inoculation. It produces abundant mycelial growth and conidia in abundance after 9 days' growth on sterilized bean pods; chlamydospore production is scant.

TEMPERATURE RELATIONSHIPS

The effect of temperature upon the growth of *Cephalosporium* was studied on potato-dextrose agar plates in constant temperature chambers. Five different isolates from celery were used. Freshly poured plates of potato-dextrose agar adjusted to pH 6.7 were inoculated by placing a small portion of agar containing the fungus in the center of the plates, after which they were incubated at the various temperatures for 21 days. Diameters of the colonies, considered the indexes of growth, were measured at the end of 7, 14, and 21 days. There was no appreciable variation in the rate of growth of the different isolates at the several temperatures. Their mean growth at the different temperatures is shown in figure 3. The optimum temperature for growth was 75° F.; there was no growth at the two extreme temperatures of 40° and 90° F.

In spore germination studies it was observed that conidia germinate readily in tap water, distilled water, 1% dextrose water, and on potato and celery-dextrose agars. Usually only one germ tube is formed from one end of each spore, but occasionally bipolar germination occurs. Maximum germination occurred in the temperature range 68°–75° F. No germination was observed at temperatures of 45° or 90° F.

FUNGICIDAL TESTS

The toxicities of Bordeaux mixture (4–4–50) and wettable sulfur (3 pounds to 50 gallons of water) to germinating spores of *Cephalosporium* were tested.

The fungicides were sprayed uniformly over a series of chemically clean glass slides. After the slides were dry they were placed in moist chambers. A spore suspension was prepared from a 14-day-old culture using sterile water, and six drops of the suspension were placed on each sprayed slide. Controls consisted of drops of the suspension on slides without the fungicide. The tests were run at a temperature of 78° F. Final counts of the relative number of germinating spores were made 48 hours after the spore suspensions were placed on the slides.

The results of five separate tests with these materials showed an average of 1.5% germination with Bordeaux mixture and of 32% germination with the wettable sulfur. The average spore germination for the controls was 83%. Bordeaux mixture was apparently much more effective in inhibiting spore germination than wettable sulfur.

The pathogen

On 2% potato-dextrose agar the pathogen forms a white, cottony to appressed growth. The mycelium is branched, septate, and produces lateral, simple to compound, continuous to septate conidiophores that taper near the apex (fig. 2*F*). In culture, short, secondary conidiophores may be produced, which occasionally branch in whorls of three or four. Conidia are formed on upright, aerial conidiophores. Conidia are continuous to one-septate, with rounded ends, elliptical, and are aggregated in spherical, grayish-white, slimy heads that disperse immediately in contact with water (fig. 2*E*). Both terminal and intercalary chlamydospores are formed in culture (fig. 2*D*).

Taxonomy

The pathogen belongs in the genus *Cephalosporium* Corda. The species causing the brown spot of celery is apparently unlike any other previously described in Saccardo's *Sylloge fungorum* (**9–14**) or descriptions of *Cephalosporium* listed by other investigators (**1–8, 16**).

In view of this and the fact that no species of *Cephalosporium* have been previously reported on *Apium graveolens* or related plants, the writers consider it a new species. Accordingly, the name *Cephalosporium apii* n. sp. is proposed.

Cephalosporium apii n. sp.[5]—Mycelial colonies in culture appressed to slightly cottony, white at first, becoming gray with chlamydospore production. Sterile hyphae hyaline, septate, branched, 2–3 μ in diameter. Chlamydospores brown to black, both terminal and intercalary, 3.6–8.8 × 6.2–9.3 μ. Conidiophores short, arising laterally from the mycelium, simple to compound, septate, tapering near the apex. Conidia hyaline, elliptical to elongate, mostly continuous, occasionally one-septate, 3.4–5.6 × 7.0–16.8 μ, borne singly but remaining in mucilaginous heads, 6–14 μ in diameter in humid atmosphere.

Habitat: Parasitic on leaves and stalks of living celery plants (*Apium graveolens*) in Colorado.

Summary

1. The *Cephalosporium* causing the brown-spot disease of celery is described. The name *Cephalosporium apii* n. sp. is proposed.

[5] Coloniis mycelialibus in culturis appressis usque subbyssoideis, primum albis, demum e chlamydosporis cinerascentibus; hyphis sterilibus hyalinis, septatis, ramosis, 2–3 μ in diametrum; chlamydosporis brunneis usque nigris, et terminalibus et intercalaribus, 3.6–8.8 × 6.2–9.3 μ; conidiophoris brevibus, e mycelio lateraliter oriundis, simplicibus vel compositis, septatis, ad apices attenuatis; conidiis hyalinis, ellipticis usque elongatis, plerumque continuis sed interdum uniseptatis, 3.4–5.6 × 7.0–16.8 μ, singulatim oriundis sed in capitulis glutinosis 6–14 μ in diametrum in aere humido perstantibus. Hab. in foliis caulibusque plantarum vivarum Apii graveolentis, Colorado.

2. When mature celery stalks, 1-year-old plants, or seedlings were inoculated with the fungus, the disease was produced in typical form. The organism was readily re-isolated in pure culture.

3. Celery was the only one of the various members of the Umbelliferae inoculated that proved susceptible to infection.

4. The organism grows readily on potato or celery-dextrose agars and on cooked celery stalks.

5. Studies of five different isolates grown on potato-dextrose agar plates at different temperatures showed that the optimum temperature for growth was 75° F. No growth was observed at the two extreme temperatures of 40° and 90° F.

The writers are indebted to Miss EDITH K. CASH, Associate Mycologist, Bureau of Plant Industry, Soils and Agricultural Engineering for preparing the Latin diagnosis.

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INHIBITORY AND STIMULATORY EFFECTS OF INDOLEACETIC ACID ON DEVELOPMENT OF THE BEAN HYPOCOTYL[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY **624**

GEORGE K. K. LINK AND RICHARD M. KLEIN

Introduction

In 1935 CZAJA (**4**), using LAIBACH's lanolin paste method, applied growth substances obtained from human urine to straightened hypocotyls 1–5 cm. long of seedlings of *Helianthus annuus*, *Pisum sativum*, and *Lupinus albus* and to the wound surface of such hypocotyls after bisection directly below the cotyledonary node. He reported on both gross behavior of the treated plants and limited histological studies of affected hypocotyls.

In 1936 BROWN and GARDNER (**2**) described tumor production by stems of Red Kidney bean and other plants following wounding and local application of 3% indoleacetic acid (IAA) in lanolin.

[1] This work was aided in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

They also recorded tumor production by bean stems following application to the cut surface of a lanolin paste containing extracts of *Agrobacterium tumefaciens*, the incitant of crown gall in plants. In the same year KRAUS, BROWN, and HAMMER (**8**) published an extensive study of the gross and histological reactions of bisected second internodes and of severed pods of Red Kidney bean to a single application of 3% IAA in lanolin.

LINK, WILCOX, and LINK (**11**) in 1937 published studies on the gross responses of various intact and severed organs of bean and tomato to wounding and to applications of IAA in lanolin paste in a wide range of concentrations. They found that inhibition and stimulation of growth and development depended upon auxin concentration and upon other factors, such as the organ treated, age of the treated area, and locus of paste application. Some effects of applications of ether extracts of cultures of *A. tumefaciens* and of IAA on cells and tissues of the intact hypocotyl were also reported. This paper was the first to point out that "the effects of this one substance (IAA) in different concentrations and amounts on the various organs of the bean illustrate practically all the major types of functional and material injuries known to the phytopathologist," later many of these cellular and tissue changes have been shown to be induced by herbicides. It also showed how its experiments and data exemplified the basic principles discussed in theoretical papers by LINK (**9, 10**) dealing with the concept of a causal complex comprising intrinsic and extrinsic factors, including animal and plant hormones.

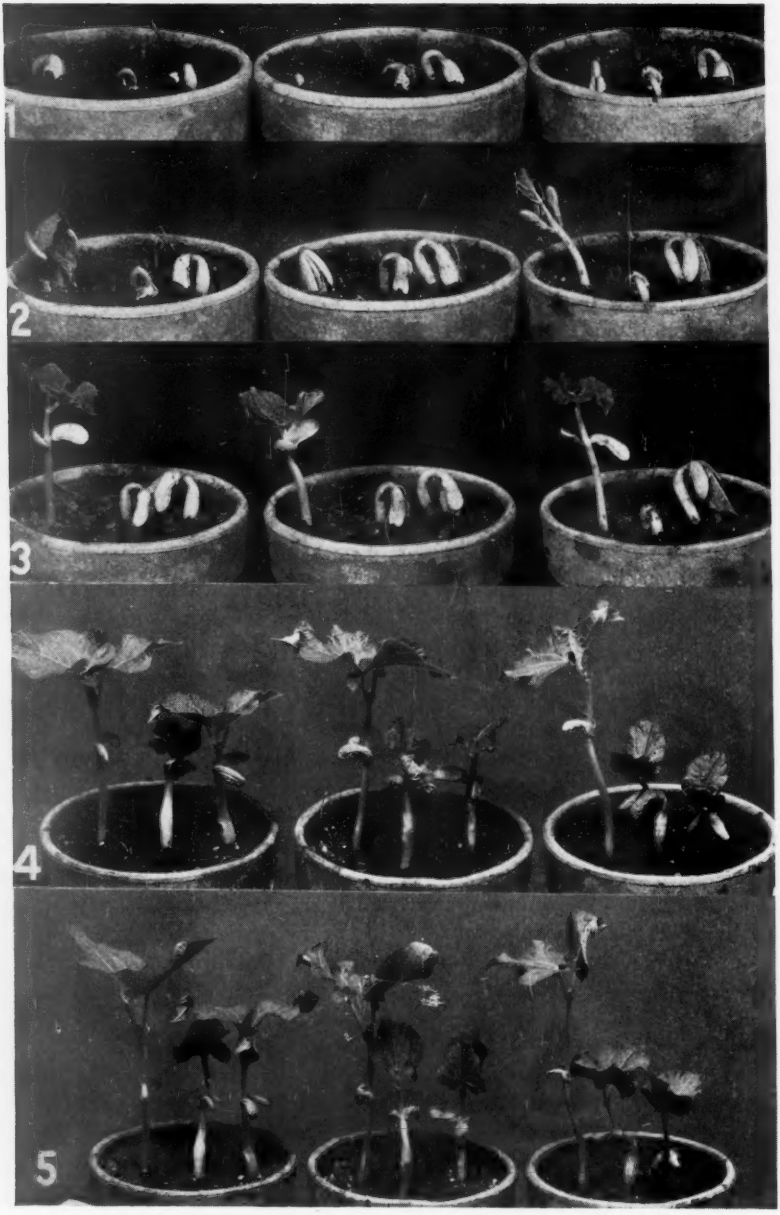
The present study, begun in 1937, was made to determine the histological changes preceding and concomitant with the gross changes induced in intact hypocotyls of bean seedlings of various ages following application of 3% IAA in lanolin. It undertook to supplement the numerous histological studies which had appeared in the literature since 1936 on the effects of IAA on various parts of the mutilated bean plant.

Material and methods

Plantings of *Phaseolus vulgaris* L. var. Red Kidney were made in loam soil at various times of year from 1937 to 1950. Unwounded hypocotyls were treated with a standard dose (0.01 ml.) of 3% IAA in lanolin at different stages of seedling development. Control plants showed no change following application of lanolin alone.

In studies of the young seedling the paste was applied when the convex side (top) of the arched seedling was 1–2 cm. above the soil line, and the cotyledons were slightly exposed. This stage was usually reached 5–6 days after planting (fig. 1). The arch, rather than regions above or below it, was selected for study because it (*a*) provides a definite locus for reference and application of paste and (*b*) consists of young tissues whose cells are in different stages of development in the convex and concave sides of the arch. Paste was applied unilaterally to convex or concave sides of the arch or equilaterally as a band around the hypocotyl at the arch. Equal amounts of paste covering approximately equal areas were applied to each plant treated unilaterally. In plants treated by banding, a greater presentation area (**7**) was necessarily produced. Observations for gross effects were begun immediately after application and were continued through 7 and 8 days, after which no new developmental changes in the hypocotyls were detected.

In an experiment set up for photographing, three pots, each containing



FIGS. 1–5.—Bean plants following treatment of arch with 3% indoleacetic acid in lanolin. (Description in text.)

three plants in a row, were used. The three pots were placed in the same position for each of the eight photographs (figs. 1–8). The plant on the left side of each pot is the control, the other two, the treated. The experimentally modified plants in the left pot were treated on the convex side of the arch, those in the center pot on the concave side, and those in the right pot by banding the arch. The first photograph (fig. 1) was taken within 30 minutes after application of the paste. Thereafter, the pots were photographed at 24-hour intervals through 7 days after application. Aside from slight differences in plant form, owing to seasonal variations in light and temperature, these photographed plants are representative of all other tests.

For studies of the straightened hypocotyl plants were selected for uniformity of development 24 hours after the arch stage and were divided into three equal groups. At this age most plants had straightened their hypocotyls, cotyledons were expanding, and primary leaves had begun to spread apart. One group of plants was treated at once, the second 24 hours, and the third, 48 hours later. Treated plants received standard doses of 3% IAA in 0.01 ml. of lanolin. Paste was applied unilaterally on the sides of the hypocotyl which had been the convex and concave sides of the arch, respectively (fig. 10). These are hereafter referred to as the "convex" and "concave" sides of both the arched and the straightened hypocotyl. Paste was also applied equilaterally by banding at the various loci. In each group paste was applied to three lots of plants, at a different locus on the hypocotyl in each lot. In the first lot paste was applied immediately below the cotyledonary node; in the second, at the middle of the hypocotyl; and, in the third, as close to the soil line as possible. In each lot an equal number of plants was treated on the convex side, on the concave side, and by banding. Adequate controls were set up for each lot. Observations were begun immediately and continued for a week.

Material for histological studies of the arched hypocotyl was collected at recorded intervals from 3 to 192 hours after application. Sample segments of hypocotyls were fixed in Navashin's fixative and were usually imbedded according to a tertiary butyl alcohol schedule. Transverse and longitudinal sections cut at 10 μ were stained by a modified Flemming triple, by the safranin-fast green, or by Conant's quadruple methods. To supplement these, freehand sections and sections cut at 25 μ with a carbon dioxide freezing microtome were made.[2]

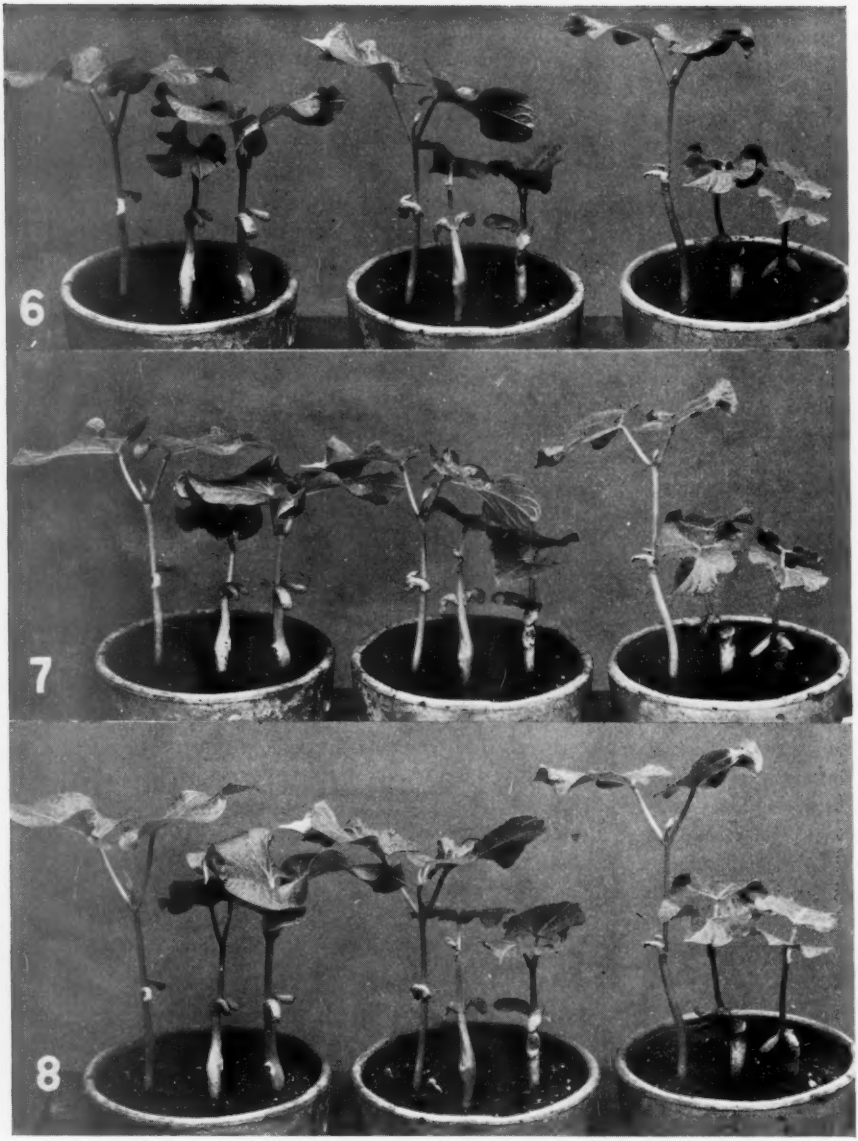
Observations

Gross responses

Behavior of arched seedlings.—Three to 5 hours after application of 3% IAA-lanolin paste, tissues directly beneath the side of application often became yellowed. With time this discoloration became more marked, the affected tissues often turning almost white within 24 hours.

Although most control plants straightened their arches and began to expand their first leaves in 24 hours, treated plants had not even pulled their cotyledons out of the soil (fig. 2). The diameter of treated hypocotyls, beginning slightly above the site of application and extending to the soil line, had increased much more than that of control hypo-

[2] Technical aid in the histological studies was supplied from July, 1937, to July, 1944, by Miss Virginia Eggers, and in 1949 by Triarch Botanical Products which imbedded, sectioned, mounted, and stained material collected and fixed by us. Figures 12–15, 20, and 21 are from slides made by Triarch; figures 16–19 from slides made by Miss Eggers.



FIGS. 6–8.—Bean plants following treatment of arch with 3% indoleacetic acid in lanolin. (Description in text.)

cotyls and was greatest from a short distance below the zone of application to the soil line (figs. 2, 11). The increase was most marked and uniform in banded hypocotyls. In the unilaterally treated, the increase was not uniform and was greatest on the treated side. Unilaterally treated hypocotyls often bend sharply just below the soil level on the treated side (fig. 2, middle pot, right plant), bending being most pronounced in plants treated on the convex side of the arch. This negative bending disappeared within 24 hours. Rate and extent of these changes varied in different lots tested from 1937 to 1950 and in plantings made at different seasons. Generally the swellings were more severe during the winter months while other changes were then slightly delayed.

In 48 hours (fig. 3) control hypocotyls had undergone considerable elongation, and first internodes also had lengthened. Hypocotyls of most treated plants had only straightened and elongated sufficiently to free the cotyledons from the soil.

In 72 hours after application (fig. 4) hypocotyls of control plants had practically completed elongation, but first leaves were not yet completely spread apart. Treated plants, by now, generally had pulled their cotyledons out of the soil, and their arches were straightening. Diameters of treated hypocotyls, beginning slightly above the presentation area and extending to the soil line, had increased much more than those of control plants. Diameter increase now was greatest at the locus of treatment, tapering off gradually down to the soil line, but above the treated area it ended abruptly. In banded hypocotyls the increase was uniform along all radii and greater than in unilaterally treated plants. In the latter the increase was not uniform along all radii but greatest on the treated side. As a result, unilaterally treated hypocotyls were more nearly elliptical in transverse section than the controls, the long axis passing through the locus of application to the opposite side (fig. 13).

Between 4 and 6 days (figs. 5–7) all treated plants had pulled their cotyledons out of the soil. By 5 days (fig. 6) definitely localized tumors were evident at the loci of application in addition to general tumefaction of the hypocotyl, the banded hypocotyls bearing the largest localized tumors. These tumors increased in size even after generalized abnormal increase in thickness had ceased. No treated hypocotyls attained the length of the controls. This observation is in conflict with the measured increase in length of treated hypocotyls reported by LINK *et al.* (11). We have not been able to find a basis for this discrepancy. Epicotyls of treated plants usually also had not developed so far as those of controls. In plants treated by banding the arch, hypocotyls were definitely malformed because of faulty straightening and large localized tumors. Roots had begun to emerge near the treated area (fig. 7).

By 7 days after application (fig. 8) treated plants were shorter than nontreated because of subnormal elongation of the hypocotyl and first internode; leaf expansion was also subnormal. Adventitious roots were just beginning to emerge from beneath the epidermis of unilaterally treated hypocotyls. In a parallel series of photographs made during the winter months, differences between control and treated plants in internodal elongation and leaf blade expansion were less marked than those evident in this series of photographs taken in May (figs. 1–8). Figure 9 is an enlarged, detailed view of a hypocotyl 7 days after banding the arch. The arch had not straightened

completely; there was a massive local tumor at the locus of application and much generalized tumefaction below it; eight rows of closely crowded roots had developed; and the outer tissues of the tumor were sloughing off.

STRAIGHTENED SEEDLINGS.—Noticeable bends developed within 3–5 hours after unilateral application of auxin paste in the upper, middle, and lower thirds of the straightened hypocotyl. In all cases the bending was negative, i.e., away from the side of application (fig. 10). Reaction was most marked in those plants treated on the convex side of the hypocotyl. The zone of hypocotyl forming the bend and the angle of bending depended mainly on age of the straightened hypocotyl and, to a lesser extent, upon side of application. Angle of bending also showed seasonal variation. Plants treated 24 hours after the arch stage reacted most, those treated 48 hours after this stage were less reactive, and little or no bending was elicited in hypocotyls treated 72 hours or more after the arch stage. Banded hypocotyls bent little, if any, when paste was applied uniformly.

FIG. 9.—Enlarged view of hypocotyl of bean in fig. 8, 7 days after banding arch with 3% indoleacetic acid in lanolin.

In plants treated 24 hours after the arch stage (fig. 10) bends occurred in the middle third of the hypocotyl, but below the locus of treatment, when application was made at the top of the hypocotyl; in the middle third and at or below the site of treatment when application was made at the middle of the hypocotyl; and in the middle third and above the treated area when application was made to the base of the hypocotyl. Hypocotyls treated at middle or top showed bends up to 90° from the vertical when treated on the convex side and up to 60° when treated on the concave side. In hypocotyls treated at the soil line bends were smaller (20°–30°), and little difference was noted between curvatures of convex and concave sides.

In plants treated after 48 hours (fig. 10) bends occurred in the upper third of the hypocotyl at the locus of treatment when application was made to the hypocotyl top; in the upper third of the hypocotyl above the site of treatment when application was made to the middle of the hypocotyl; and no bends occurred when application was made to its base.

No difference in angle of bending following application on the convex and concave sides was noted. The angle of bending in this group was less than that of plants of the 24-hour group.

No bending of the hypocotyl was noted in the group of plants treated 72 hours (fig. 10) after the arch stage. Negative bending (40°–50°) occurred in the first internode, however, irrespective of side of application when paste was applied just below the cotyledonary node.

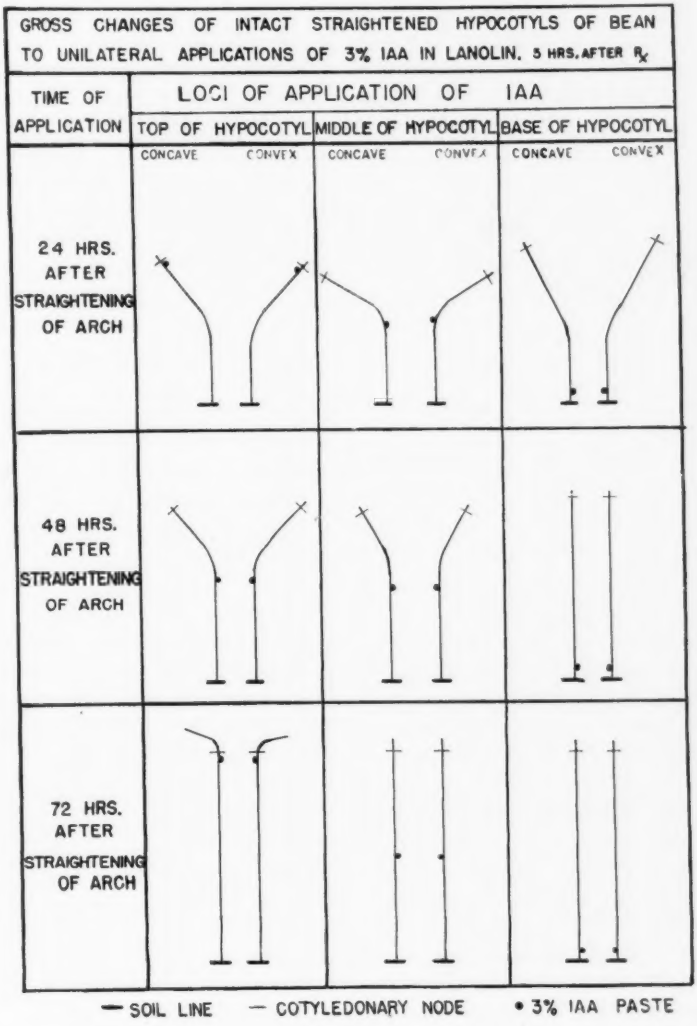

GROSS CHANGES OF INTACT STRAIGHTENED HYPOCOTYLS OF BEAN TO UNILATERAL APPLICATIONS OF 3% IAA IN LANOLIN. 5 HRS. AFTER Rx
TIME OF APPLICATION
LOCI OF APPLICATION OF IAA
TOP OF HYPOCOTYL
MIDDLE OF HYPOCOTYL
BASE OF HYPOCOTYL
CONCAVE
CONVEX
24 HRS. AFTER STRAIGHTENING OF ARCH
48 HRS. AFTER STRAIGHTENING OF ARCH
72 HRS. AFTER STRAIGHTENING OF ARCH
— SOIL LINE
— COTYLEDONARY NODE
• 3% IAA PASTE

FIG. 10.—Diagrammatic representation of changes induced in straightened bean hypocotyls by unilateral applications of 3% indoleacetic acid in lanolin to various loci on convex and concave sides of hypocotyl at different stages of elongation.

Hypocotyls in each set were marked with India ink at 2-mm. intervals on both convex and concave sides along their entire length and measurements were made before and after application of IAA. In all instances of bending there was more than normal elongation on the treated side and less than normal on the opposite side. These changes, without exception, were restricted to that portion of the hypocotyl which was in the normal process of final elongation at the time of application.

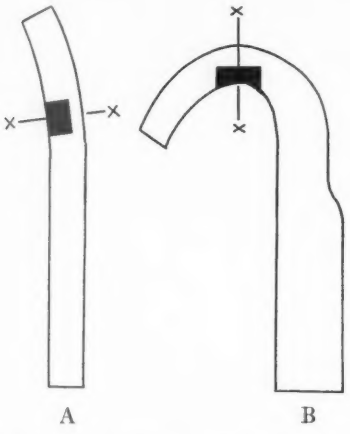


FIG. 11.—Diagrammatic representation of bean hypocotyls (fig. 2) severed at ground line and at cotyledonary node 6–7 days after planting, and 24 hours after application of IAA; *A*, control plant; *B*, plant treated on convex side of arch. Transverse sections of figs. 12 and 13 were cut along lines *x*–*x*. Sites of longisections of figs. 14 and 15 are indicated by blackened areas of *A* and *B*, respectively.

Within 24 hours after application the angle of bending diminished, but complete straightening was not attained throughout the period of observation. Ultimately, the amount of elongation on both sides was slightly deficient relative to elongation of comparable nontreated hypocotyls.

These bends and almost complete subsequent recovery therefore probably are due to both an accelerated and finally slightly deficient elongation of cells of the treated side and to retarded and ultimately greater deficient elongation of cells of the opposite side. In banded hypocotyls no final excessive elongation was evident; in fact, at times, elongation seemed to be deficient.

Tumors began to form within 4–5 days at each locus of application and developed as those described for plants treated when in the arch stage. Rate and extent of tumor development depended on hypocotyl age at time of application. These tumors were more sharply restricted to areas of paste application than those on arched seedlings, and there was little involvement of tissues above or below them. Bending and tumor formation occurred at the same site only when bending had taken place at the locus of application. In banded hypocotyls tumors always occurred at the treated areas and, as in arch-treated hypocotyls, were more massive and involved the entire circumference.

HISTOLOGY

ARCHED SEEDLINGS.—Frozen sections of treated regions made within 4–5 hours after application of auxin paste showed "ghosts" of chloroplasts present in normal position, but chlorophyll had begun to disappear from the epidermis and cortical parenchyma. KRAUS *et al.* (**8**) reported destruction of chloroplasts in wounded treated bean material, but LINK *et. al* (**11**) found only differential chlorophyll loss in unwounded but treated bean and tomato hypocotyls. KLEIN and LINK (**7**) reported the same type of chlorophyll destruction in decapitated flax hypocotyls treated with 2,4-dichlorophenoxyacetic acid. The present study also confirms the report (**11**) that

large intercellular spaces and schizogenous cavities, formed in the cortex as a result of abnormal radial and tangential cell enlargement, contribute to whitening of treated areas.

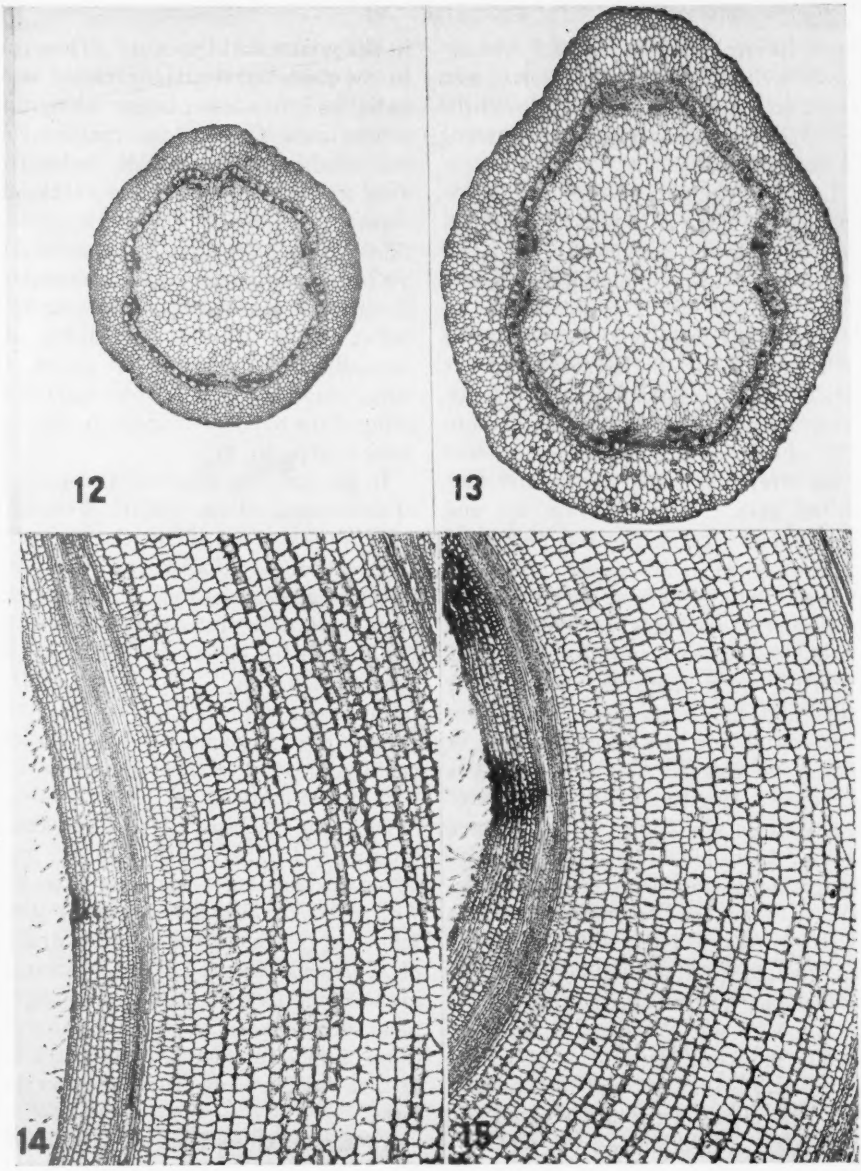
Longitudinal sections of a normal hypocotyl 6–7 days after planting (fig. 14) showed that cells previously composing the concave side of the arch had almost completed the primary elongation involved in straightening the arch. The arch had almost disappeared, and the hypocotyl was practically erect. In transverse section (fig. 12) parenchymatous cells of the former arch zone showed equal stretching along radial and tangential axes. Transverse (fig. 13) and longitudinal sections (fig. 15) of treated hypocotyls 24 hours after application showed that the normal pattern of cell enlargement was lacking. Parenchyma cells of cortex, rays, and pith, especially of cortex, had stretched excessively along radial and, to a lesser extent, along tangential axes but had elongated little or none along axes parallel to the long axis of the stele (longitudinally). This change in pattern of stretching along the three axes is responsible for delay in hypocotyl straightening, for delay and final deficiency in its over-all elongation, and for its abnormal thickening. Cells below the soil line had lengthened along the longitudinal axis more on the treated than on the opposite side, causing the negative bending observed (fig. 2).

Transverse (fig. 17) and longitudinal (fig. 19) sections of the straightened but treated hypocotyls made from material fixed 72 hours (fig. 4) or later after application also showed that the eventual straightening was due to an abnormal pattern of stretching along the three axes of affected cells. Cells on the convex side had lengthened along the longitudinal axis but less than similarly situated cells in the nontreated hypocotyl. They continued abnormal stretching mainly along radial and, to a lesser extent, along tangential axes. Cells on the concave side elongated less than normally in longitudinal axes but sufficiently to equal the deficient stretching of cells on the convex side. They also stretched excessively mainly along radial and to a lesser extent along tangential axes. Excessive stretching of cells in the cortex had led to abnormally large intercellular spaces, to schizogenous cavities, to abnormal thickening of the hypocotyl, and to tumor formation (figs. 20, 21).

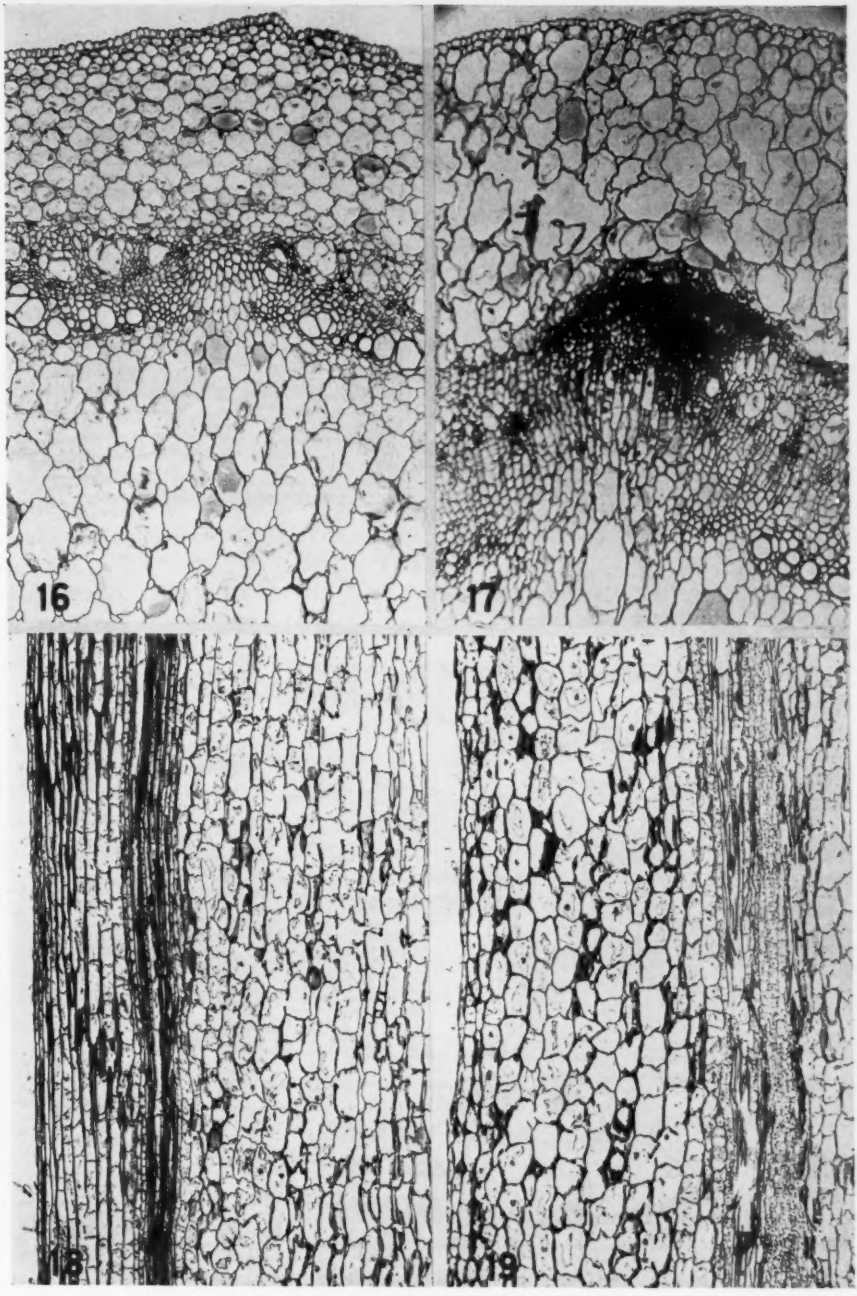
In general, the sequence and pattern of subsequent cellular and tissue changes —cell division, cell differentiation, meristem and adventitious root formation— in the treated intact hypocotyl were the same as those reported by KRAUS *et al.* (**8**) for bisected second internodes of the bean treated with 3% IAA. Effects in the intact organ, however, were not so rapid, extensive, or pronounced. Also, neither cell divisions nor tracheid differentiation occurred in cortical, ray, or pith parenchyma as in the bisected, treated second internode. In this respect, effects produced in the intact hypocotyl treated with 3% IAA also differed from those following treatment with ether extracts of *A. tumefaciens* (**11**). Rates of change in the intact, treated second internode, as reported by BEAL (**1**), were the same as those in the intact, treated hypocotyl. In neither was there any proliferation in the pith.

Abnormal cell division directly under the treated area in the endodermis initiated a meristem to which both pericycle and phloem contributed. Histological details of development of this meristem and of adventitious roots also have been reported (**8**) and need not be repeated here.

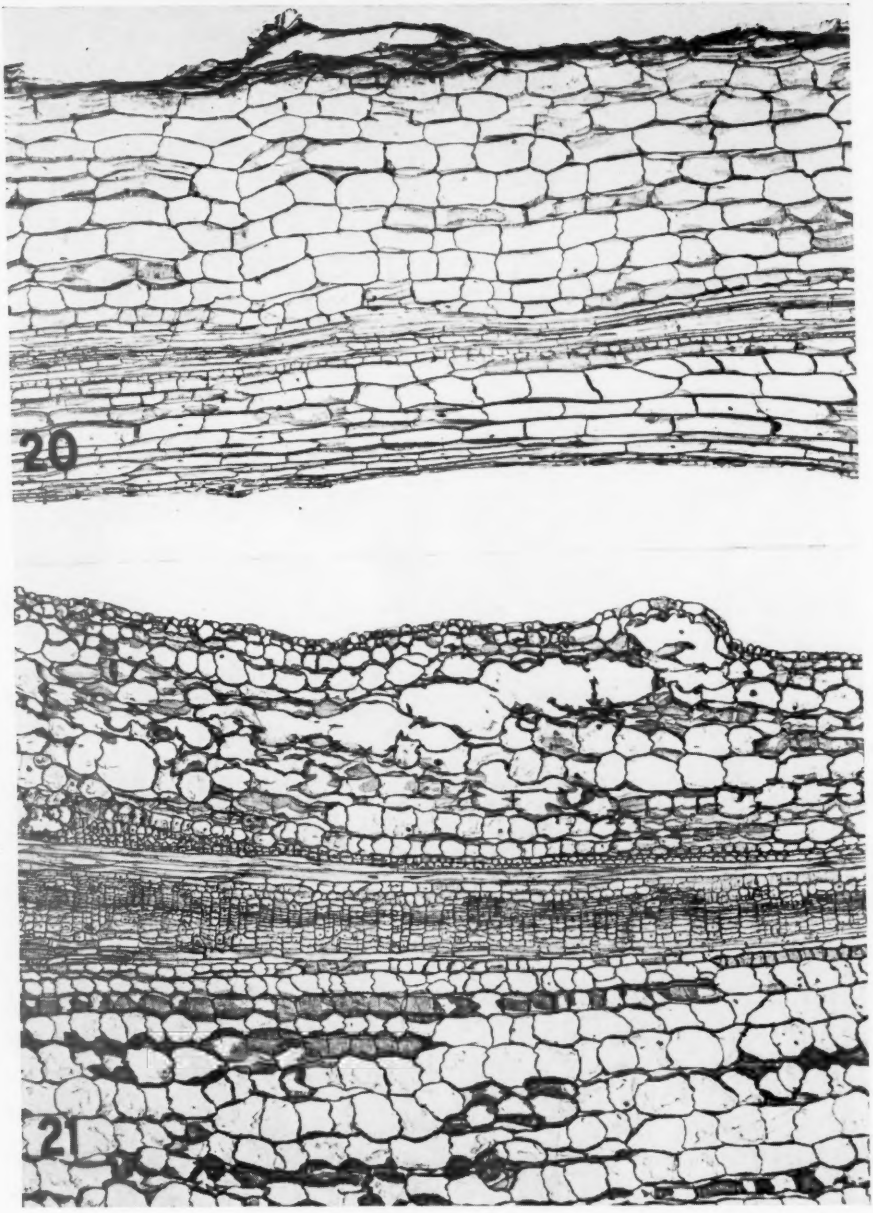
The pattern of cell changes in the



FIGS. 12–15.—See fig. 11. Figs. 12, 14, untreated; figs. 13, 15, treated. Magnification of figs. 12, 13, ×25; figs. 14, 15, ×11. Tops of figs. 12 and 13 are tops of convex side of arch. Increase in diameter of treated hypocotyls is due to excessive enlargement of cells along their radial and tangential axes; delay in straightening is due to deficient elongation of cells of all tissues at the levels of application, and to delay of elongation of cells of the concave side, along the longitudinal axis. The difference in diameter of control and treated plants after 24 hours is not always so great as that shown in figs. 12 and 13, or as slight as shown in figs. 14 and 15.



FIGS. 16–19.—Sections of hypocotyls in same stage of development as those of fig. 4 (72 hours after application) cut at level of IAA-paste application. Figs. 16, 18, control; figs. 17 and 19, treated plants. All at same magnification. Figs. 16 and 17 show that diameter increase in treated plants was due to cell enlargement along radial and tangential axes, to formation of schizogenous cavities in cortex, to anticlinal and periclinal cell divisions in regions between endodermis and pith, and to formation of adventitious root meristems. Figs. 18, 19 show that the treated hypocotyl is characterized by deficient elongation of cells along longitudinal axis and by excessive stretching along radial and tangential axes.



FIGS. 20, 21.—Longitudinal sections of hypocotyls in same stage of development as those of fig. 7 (144 hours after application). Fig. 20 shows tissues between epidermis to pith cavity which characterize normal bean hypocotyls of this age. Fig. 21, cut at base of tumor, shows tissues from epidermis to part of intact pith which characterize treated plants of this age. In addition to radially enlarged cortical and pith cells and cortical cavities in region between endodermis and protoxylem, there are marked increases in cell numbers which contribute to tumor formation.

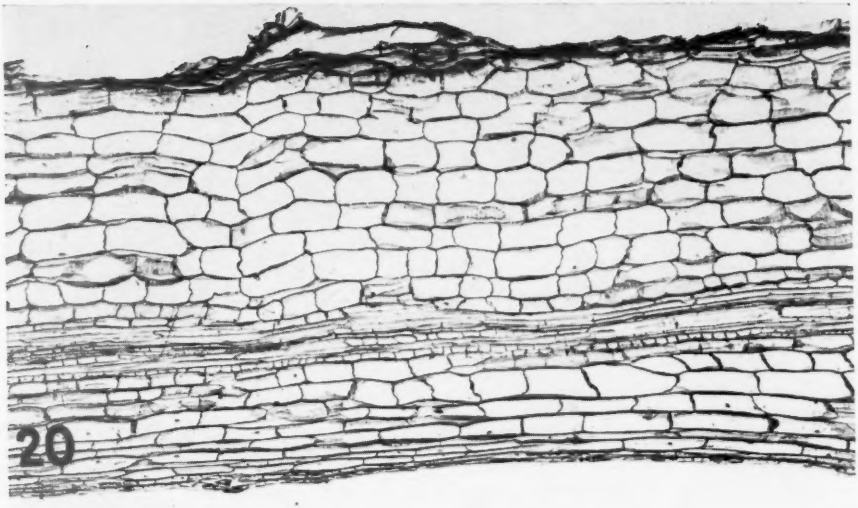
banded hypocotyl was essentially that of the unilaterally treated organ. However, cells along all radii were affected and most severely on the concave side. Elongation along the longitudinal axis was more strongly inhibited than in unilaterally treated hypocotyls, and stretching along radial and tangential axes was more pronounced, leading to more massive general and localized tumefaction. Development of adventitious root meristems was not restricted to the area directly beneath the locus of treatment but extended for some distance above and below it, as did the area of most pronounced local swelling. Further, formation of adventitious roots from the meristem proceeded more rapidly than that induced by unilateral treatment.

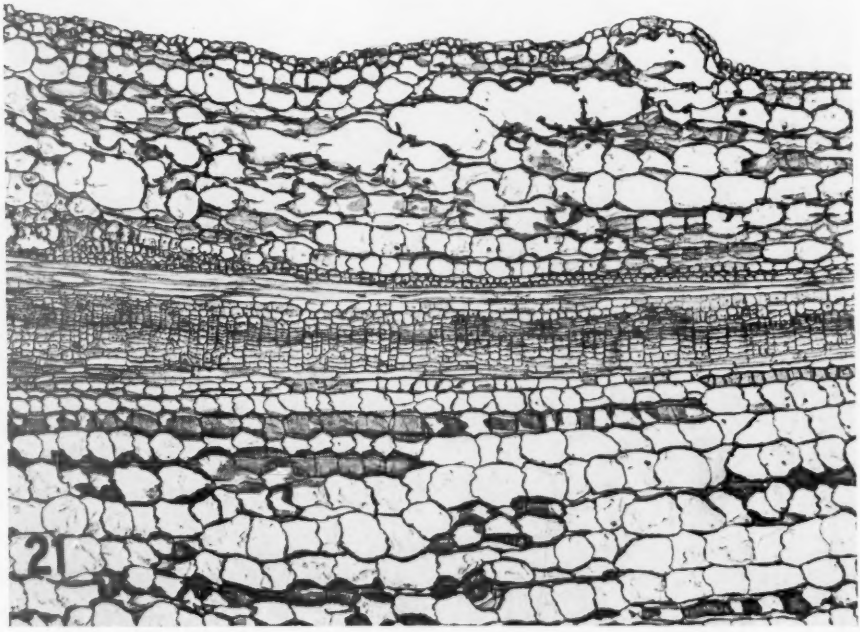
STRAIGHTENED SEEDLINGS.—Longitudinal and transverse sections of the zone of bending 18 hours after application showed that the bending was due solely to excessive cell lengthening along the longitudinal axis on the treated, relative to the opposite, side. Neither cell divisions in any plane nor stretching along radial and tangential axes were noted at this time. Stretching along these axes was noted later at the loci of application, but not at site of bending if this was not also the locus of treatment. Here, too, these changes in stretching along radial and tangential axes inaugurated sharply localized tumor formation.

Discussion

The development of the normal seedling appears to be essentially similar to that of the flax seedling described in detail by CROOKS (3) and LINK and EGGERS (12). In the mature bean seed the hypocotyl is slightly bent below the cotyledonary node. Later, during early growth stages, the hypocotyl attains the arch shape because of an initial differential elongation along the long axis of the stele of cells on the convex side of the initial curvature relative to those on the concave side, the former elongating, the latter not. Four to 6 days after planting the cells on the concave side, not previously elongated, also undergo lengthening. By this integrated sequence of differential elongating the hypocotyl is able to pull its cotyledons out of the soil and to straighten the arch. There is no indication that cell division plays any role in these stages of development. Our experiments indicate that the cotyledons or substances within the cotyledons, but not the epicotyl, are causally related to the process of straightening. When arched seedlings were bisected beneath the cotyledonary node, straightening and elongation were delayed for several days. This observation is in harmony with that of JACOBS (6). The eventual straightening, however, is not dependent upon callus formation on the wound surface. The relation of this finding to regeneration of a "physiological tip" in the bisected *Avena* coleoptile deserves further investigation.

The final phase of cell elongation is initiated 5–6 days after planting while the hypocotyl is still in the arch stage. It originates at the hypocotyl base and progresses toward the cotyledonary node, cells in the elongating zone practically attaining mature length before cells in the next prospective zone of stretching begin final elongation. When this wave of elongation reaches the zone of the straightened arch, cells on both convex and concave sides undergo equal elongation along the longitudinal axis. Finally, the zone of elongation passes into the region above the arch. In the main, the upper third of the elongated hypocotyl is derived from that portion in the young hypocotyl which was above the arch; the





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middle third, from that portion which formed the arch; and the lower third, from the portion which was below the arch.

This study has shown that application of a 3% IAA paste retards and in part inhibits attainment of this normal pattern of hypocotyl and epicotyl development and leads to abnormal curvatures and to tumor formation in the straightening and straightened hypocotyl. It also has established that the first histological change detectable is derangement of cell enlargement which is the basis of normal gross development and of the size and form of the hypocotyl.

The cellular responses noted are functions in part of the concentration of IAA effective in the cells, in part of the capacity of the cells to be affected and to react. These in turn seem to be functions of the stage of development of the cells and of their past history. The complex of internal and external factors involved has been discussed by LINK and co-workers (9, 10, 11).

The relative roles of concentration of IAA and the stage of development of affected cells in the effects noted are difficult to evaluate because the concentration of IAA effective in the cells is not determinable. The normal (usual) and functional (physiological) concentration of IAA in the various cells of the bean hypocotyl is not known. Neither is it known what abnormal (unusual) concentrations are dysfunctional (pathogenic). It is not known how much applied IAA enters the treated organ or for how long it enters following an application, or how the developmental stage of the epidermis and other cells affects entry and passage of the applied IAA. Consequently, it is not even known whether at the same locus of application with 3% IAA, but at different stages of development, the cells are affected by the same concentration of IAA. Presumably more IAA entered the banded than the unilaterally treated hypocotyls, presumably more entered the arched than the straightened hypocotyl, and presumably the concentration of IAA in the cells was greater at the loci of application than elsewhere in the hypocotyl, less below it and least above it.

Irrespective of the stage of cell development at the locus of application, applied 3% IAA led to such a concentration of IAA in these cells that they stretched excessively along radial and tangential axes. A range of concentration of considerable latitude may be involved. Given a concentration within this range, stretching was most pronounced within young cells of the arch region and least pronounced in fully elongated cells of straightened hypocotyls. Simultaneously this concentration initially inhibited elongation along the longitudinal axis in the former and did not affect elongation in the latter. These findings indicate that the effect produced by a given concentration of IAA is in part a function of the stage of cell development.

In the straightened but elongating hypocotyl negative curvatures occurred in the zone of elongation of the treated side, irrespective of whether the locus of application was in, above, or below this zone, provided this zone was not too far above the presentation area. Only when the zone included the place of application did curvature occur at the locus. Under these circumstances the concentration of IAA attained in the affected cells produced accelerated and, ultimately, slightly deficient elongation along the longitudinal axis, irrespective of the side treated, and excessive stretching along radial and tangential axes. In cells of the opposite side elongation along the longitudinal axis was temporarily retarded

and finally deficient. Presumably in each instance the IAA concentration was less in these cells than in elongating cells of the treated side. We have no explanation of the observation that the same cells when on the treated side temporarily elongate excessively in rate and amount and when opposite the treated side, presumably containing a lower concentration of IAA, elongate deficiently in rate and amount.

The same problem is presented when curvature takes place above or below the locus of application, with an additional problem posed by the observation that the cells which undergo abnormal differential elongation along the longitudinal axis on opposite sides of the hypocotyl—thus causing the negative curvature—do not stretch abnormally along radial and tangential axes, while cells at the locus of application do not experience this abnormal pattern of elongation along longitudinal axes but do stretch abnormally along radial and tangential axes. What the possible roles of IAA concentration and stage of development are in these situations we are unable to postulate.

If it is assumed that the concentration gradient of IAA from the IAA paste into the area under consideration remains constant throughout the period of observation, the eventual more or less complete straightening of treated hypocotyls must occur while the effective IAA concentration is essentially the same as when cell elongation was initially inhibited or stimulated. Therefore, the eventual but deficient elongation along the longitudinal axis which straightens the arch or eliminates much of the curvature of the bent hypocotyl appears to be a function of both the past experience of the affected cells and of their stages of development. How the same concentration of IAA can simultaneously affect differentially the stretching of a cell along its three axes, always affecting it in the same manner along radial and tangential axes but not always—and differently in extent when it does—along the longitudinal axis, raises an interesting problem in the mechanism of cell enlargement and the roles of IAA in this enlargement.

These observations also pose problems relative to the direction of IAA movement in the treated hypocotyl. Apparently under certain conditions there is enough upward movement of IAA to produce concentrations of IAA which will induce negative curvatures of considerable magnitude in cells above the locus of application in zones of elongation of hypocotyl and first internode.

Applications of 3% IAA also led to a lack of normal correlation in differentiation of the tissue systems of the hypocotyl. DOUTT (**5**) reported that the hypocotyl of normal bean seedlings is positively disposed to form adventitious roots from the lower portion and that these roots arise opposite one or more of the protoxylem points and are derived solely from the pericycle. In hypocotyls treated with IAA, however, adventitious roots arise opposite the rays in meristems which originate in the endodermis. Later, the pericycle and phloem become active and contribute to them. This indicates that IAA is capable of inducing meristematic activity in tissues which have passed beyond the stage in which, without extrinsic stimulation, they normally form meristems, and of so changing the reactive disposition of cells, which under normal conditions do not contribute to a meristem, that they do contribute to a meristem initiated by another tissue.

KRAUS *et al.* (**8**) reported divisions in cortical and pith parenchyma with some differentiation of tracheids in bisected second internodes of the bean treated on

the wound surface with 3% IAA in lanolin. LINK *et al.* (11) reported the same for intact hypocotyls of the bean treated with ether extracts of cultures of *A. tumefaciens*. The observation that bacterial extracts elicit tracheid development in the tissues of intact tomato and bean hypocotyls (such as occur in crown gall incited by *A. tumefaciens*), and that IAA does not, in all likelihood means that the bacteria produce other growth substances in addition to indoleacetic acid (11). The observation that 3% IAA paste applied to the wound surface of a bisected second internode of the bean induces tracheid development while the same concentration applied to the intact hypocotyl does not may mean that (*a*) the reactive capacity of cells of the hypocotyl and of the second internode are different relative to equal concentrations of IAA or that (*b*) these capacities are equal but that greater amounts of IAA enter cells of the bisected organ because of ready entrance through the wound, leading to a higher concentration of IAA in the affected cells.

Summary

1. Study was made of histological changes preceding and concomitant with observed gross inhibitions and stimulations induced in the Red Kidney bean seedling (*Phaseolus vulgaris*) following unilateral and band applications of 3% indoleacetic acid–lanolin paste to various loci of the hypocotyl in various stages of straightening and elongation.

2. Within 24–48 hours after application of IAA to the arched seedling gross changes include discoloration of tissues, temporary inhibition of straightening and elongation of the hypocotyl, and excessive increase in its diameter at, below, and, to a slight extent, above the treated area. Following application the cells of the concave side of the seedling arch at first stretch along radial and tangential, but not along the longitudinal, axes. This change in the normal pattern of orientation of the axes is responsible for delay in straightening and for deficient hypocotyl elongation and, at this stage, is the main factor in abnormal increases in diameter and in localized tumor formation. Eventual straightening of the treated hypocotyl and its finally deficient elongation are due to lengthening along the longitudinal axis which is less than in untreated hypocotyls but eventually equals the depressed elongation of cells on the convex side of the arch. Later both sides lengthen in the final phase of elongation. The abnormal increase in the hypocotyl volume at, below, and, to a slight extent, above the locus of application is due to increases in the cell volume and to enlarged intercellular spaces, formation of cortical schizogenous cavities, and to root meristems arising in the tumor opposite one or more rays.

3. When IAA paste was applied unilaterally to various loci on the straightened hypocotyl 24–48 hours after the arch stage, negative bending of the hypocotyl was induced within 3–6 hours after application. Bends always occurred in the hypocotyl zone which was in process of elongating to mature length at the time of application. Curvatures were at, below, or above the treated area depending on the relative positions of the zone of elongation and of locus of treatment, provided the former was not too far above the latter.

4. Unilateral applications of 3% IAA to various loci on the straightened hypocotyl 72 hours after the arch stage did not induce hypocotyl bending but did lead to tumor development at each locus. If the treated area was directly below the cotyledonary node on either the convex

or the concave side, negative bending occurred in the zone of elongation of the first internode.

5. There is some evidence that IAA, or some substance activated by it, is capable of moving against the normal polarity gradient and under proper conditions can affect tissues above the site of application.

6. Histological studies of the bends indicated that they probably were entirely due to elongation which temporarily is excessive in rate and finally slightly deficient in amount in the cells on the treated side, and initially is deficient in rate and ultimately more deficient in amount in the cells on the opposite side. Changes in the normal pattern of cell enlargement along the three axes probably are functions of the IAA concentration in, and of the developmental stage of, the affected cell. Tumors formed at the locus of application in straightened hypocotyls treated with 3% IAA paste 24, 48, and 72 hours after the arch stage have their origin in excessive cellular stretching mainly along radial and less extensively along tangential axes. Later, cell divisions set in, and tumors develop as in treated arched hypocotyls.

DEPARTMENT OF BOTANY
UNIVERSITY OF CHICAGO

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EFFECTS OF INDOLEACETIC ACID UPON GROWTH AND RESPIRATION OF KIDNEY BEAN[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 625

BURLYN E. MICHEL

Introduction

Numerous investigations have been made of morphological and physiological effects induced by indoleacetic acid (IAA) applied to various parts of the bean plant either in a lanolin carrier or as an aqueous solution. Cuttings, plants transected at different levels, plant parts, and intact plants treated at different loci have been used in such studies (18).

Young hypocotyls of intact plants were chosen for this study because application of an IAA-lanolin paste rapidly induces a marked response without the complicating effects of a cutting injury and because an abundance of uniform material readily adaptable to respiratory measurements can be obtained. LINK *et al.* (10) first reported some of the gross morphological effects of such treatment. Later LINK and KLEIN (9) made a detailed histological study of the treated hypocotyls.

This paper reports (*a*) a study of changes in growth and development as related to respiration of the bean hypocotyl following such application and (*b*) correlation of these findings with previously observed morphological and physiological changes.

Material and methods

GREEN PLANTS

Seeds of *Phaseolus vulgaris* var. Red Kidney were selected for uniformity and planted in flats of soil in the greenhouse. When hypocotyl arches were about 1 cm. above the soil surface, a zone about 10 mm. long at the top of the arch was delimited by two lines with India ink, and only this section was used experimentally. About 10 mg. of IAA-lanolin paste were placed over an area of 15–20 sq. mm. centered between the India-ink marks on the convex side of the top of the arch.

The IAA-lanolin paste was prepared by weighing proper amounts of IAA (Merck) and U.S.P. anhydrous lanolin into a small glass vial, warming until the lanolin was soft, and then vigorously stirring the IAA into the lanolin. As microscopic examination showed, this produced an even suspension of IAA crystals and small air bubbles throughout the lanolin. Most investigators have used this or similar methods to produce such a suspension for application to plants. Concentrations lower than 0.01% were prepared by serial dilution.

The lanolin used did not affect the growth or respiration of control plants. Consequently, in most experiments control plants were completely untreated.

Plants were harvested at the times indicated in tables and figures, the IAA-lanolin paste removed with cleansing tissue, and plants or plant parts rinsed in running tap water.

Respiration was measured with standard Warburg apparatus. Freehand slices, 0.5–0.7 mm. thick, were collected and mixed in a small volume of 0.02 *M* phos-

[1] This work was supported in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

phate buffer at pH 6. Since it seemed advantageous to measure respiration as quickly as possible after harvesting and because untreated slices were little affected by washing, no washing period was employed. The slices were drained and placed in 25-ml. vessels with 5 ml. of buffer. Unless specified otherwise, the following conditions obtained during measurement of oxygen uptake and aerobic carbon dioxide release: temperature, 25° C.; pH 6; 0.02 *M* phosphate buffer; gas phase, air; 0.15 ml. of 15% potassium hydroxide in the center cup or a side arm for absorption of carbon dioxide; rate of shaking about 110 strokes per minute; and length of experiment, 2 hours. Respiratory quotients were determined by using vessels with two side arms. In one was placed 0.5 ml. of 3 *N* hydrochloric acid; in the other, 0.15 ml. of 15% potassium hydroxide free of carbon dioxide. At the end of the period for measuring oxygen uptake both acid and alkali were tipped into the main compartment to kill the tissue and to release the carbon dioxide so that it could be measured. Immediately after the first reading duplicate vessels were tipped to obtain initial carbon dioxide values. It was necessary to correct for dry-weight loss of acid-treated slices by multiplying by a factor of 1.3. Conditions for measurement of anaerobic carbon dioxide release were as above with three exceptions: the buffer was 0.01 *M* bicarbonate in 50% Ringer solution; the gas phase was 95% nitrogen and 5% carbon dioxide; and no potassium hydroxide was used.

The tissue was recovered, dried to constant weight at 80°–85° C., and weighed. Rate of gas exchange is expressed by the number of cubic millimeters of oxygen taken up or of carbon dioxide given off per milligram of dry weight per hour (QO_2 or QCO_2). The reason for using dry weight rather than nitrogen as a basis is given later. Used alone, "respiration" refers to oxygen uptake.

Nitrogen determinations, not of recovered slices, but of duplicate unsliced samples dried and ground in a Wiley mill to pass a standard 20-mesh screen, were made following standard micro-Kjeldahl procedures. Soluble nitrogen was extracted with water, the proteins being precipitated with trichloroacetic acid at the final concentration of 10%. No attempt was made to reduce nitrate nitrogen. The amount of nitrate nitrogen in green hypocotyls was found by STUART (15) to be negligible.

Ethyl alcohol was determined by a modification of NICLOUX's method (13). Lactic acid determinations were attempted by the method of BARKER and SUMMERSON (2) but without success because an interfering grass-green color formed upon addition of the p-hydroxydiphenyl reagent.

ETIOLATED PLANTS

Most of the work reported in this paper was done with etiolated plants because of difficulties in obtaining samples from green plants of sufficient uniformity to give significance to small changes in weight, volume, and length, and because of added complications from photosynthesis and diurnal variations. The material and methods were those listed above with the following exceptions and additions.

Etiolated plants were grown in torpedo sand in a dark room with a temperature range of 23°–29° C. and watered exclusively with tap water. Plants were treated and harvested in the weak red light of a partially shielded 40-watt G.E. red fluorescent lamp.

When hypocotyls were 2–3 cm. above the sand surface, three India-ink lines were marked on the straight portion immediately below the convex side of the arch by means of a threaded jig. This delimited two zones each 8 mm. long (fig. 1, *B* and *C*), the upper zone being the site of application.

The suspension of IAA crystals throughout lanolin described above was not particularly satisfactory, especially in concentrations as high as 10% IAA. Etiolated plants were treated with IAA-lanolin paste prepared by adding a small amount of peroxide-free ether to the IAA and lanolin weighed into a vial, stirring until both were dissolved, evaporating the ether *in vacuo*, and again mixing. Microscopic examination showed no crystals or other particles of IAA.

For studies involving the entire plant it was divided into five parts: epicotyl—all above the cotyledonary node; cotyledons; upper hypocotyl—that portion between the cotyledonary node and the upper India-ink mark (fig. 1, *D*); lower hypocotyl—that portion between the upper India-ink mark and the uppermost root and including the site of application (fig. 1, *A*); and roots, i.e., the rest of the plant.

Oxygen uptake of unsliced pieces of different parts of etiolated plants was measured. The pieces were placed in vessels containing two layers of filter paper plus 0.5 ml. of water. Into the center cup 0.15 ml. of 15% potassium hydroxide was placed. Oxygen uptake was measured at 25° C. in the water bath without shaking. A comparison of air and pure oxygen as gas phases showed a slightly higher (2%–7%—not significant) rate of oxygen uptake with pure oxygen. This indicates that the rate of diffusion of oxygen into the tissue was not a limiting factor with air as the gas phase.

After collection and washing, plants used for weight, volume, and length measurements were cut into the appropriate sections. To help remove adhering sand particles, the roots were immersed in a saturated sodium chloride solution for 30 seconds and then rewashed. Surface moisture of all parts was removed by blotting with paper toweling and/or cleansing tissue. Samples were placed in weighing bottles or tared stoppered Erlenmeyer flasks, weighed for fresh weights, measured by water displacement for volumes, measured for lengths (curved segments of the upper hypocotyl were measured around the outer curve by means of a graduated string), placed in tared crucibles or beakers, killed in an oven at about 110° C., dried to constant weight at 80°–85° C., and weighed for dry weights.

Results

Green plants

As reported by Link and co-workers (**9, 10**), among the first gross morphological effects of treatment of hypocotyls of green plants with 3% IAA in lanolin is a severe inhibition of hypocotyl elongation and straightening coupled with enlargement, greatest at the site of application but extending to soil level as well as slightly above the area of application. Until the second or third day this diameter increase results primarily from radial and tangential enlargement of the cells. After this time cell division plays an increasing role. After 7 days adventitious root meristems are beginning to push through the epidermis. Perhaps the only real toxic effects noted following IAA application were yellowing and then whitening of cortical layers at the treated site, with softening and eventual separation of epidermis and cortex from the lower layers as indicated by the

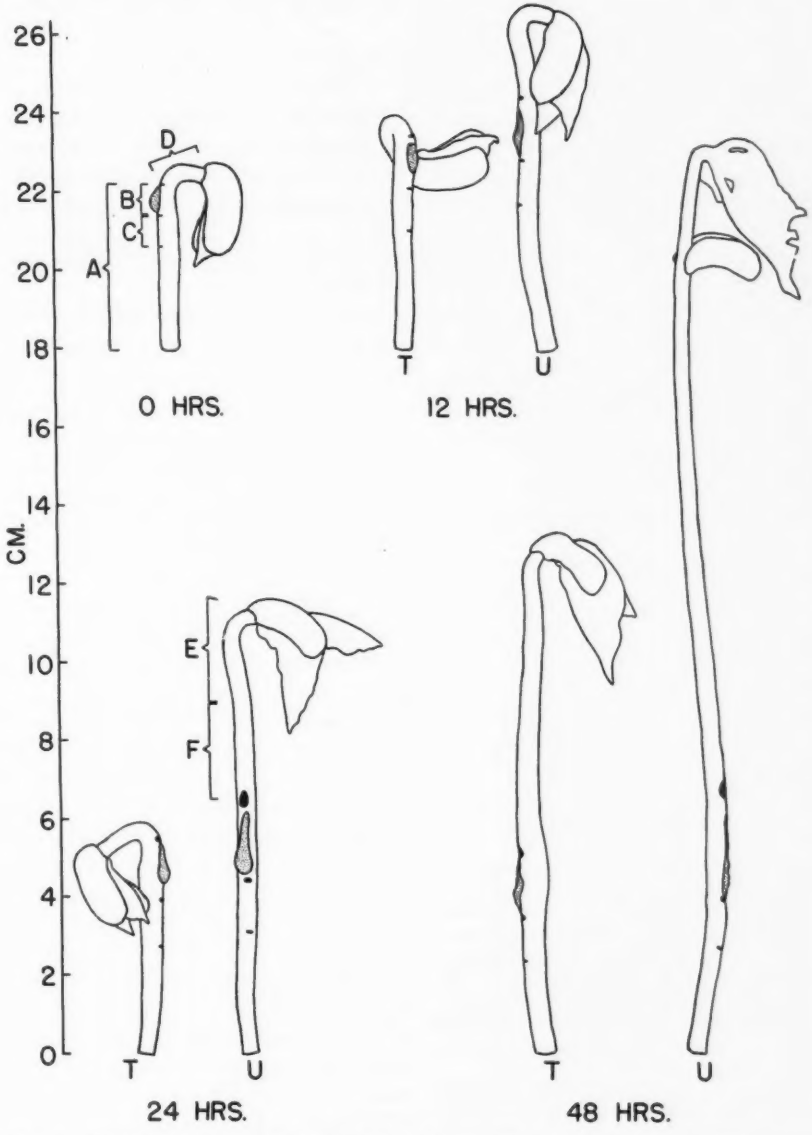

FIG. 1.—Etiolated beans treated with 1% IAA in lanolin (T) and with pure lanolin (U) 0, 12, 24, and 48 hours after application showing only that portion above the uppermost root. A = lower hypocotyl, B = upper 8-mm. marked zone and site of treatment, C = lower 8-mm. marked zone, D = upper hypocotyl, E = upper half of upper hypocotyl, and F = lower half of upper hypocotyl.

ease with which these parts could be rubbed or peeled off 4–5 days after application.

Comparison of response by the treated portions of green and etiolated plants indicates similar changes in weight, volume, and length, though of a different magnitude. Measurements made for

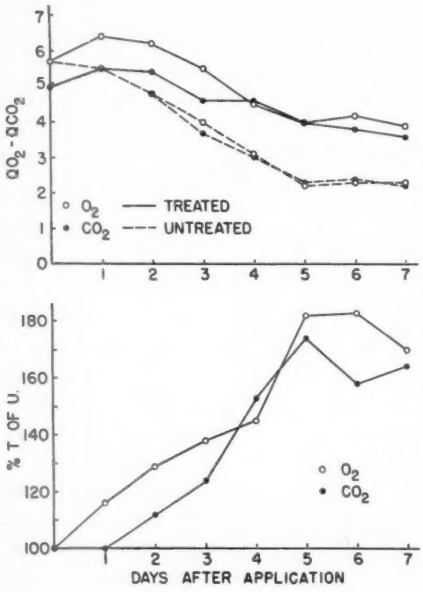

FIG. 2.—*Upper:* cu. mm. of oxygen uptake and of aerobic carbon dioxide release per mg. dry weight per hr. (QO_2 and QCO_2) of slices from green bean hypocotyls at various times following unilateral application of 3% IAA in lanolin (treated), or untreated. *Lower:* as above, with oxygen uptake and carbon dioxide release of treated hypocotyl slices expressed as percentage of that of untreated (% T of U).

etiolated plants are reported later in this paper.

Twenty-four hours after application of 3% IAA in lanolin the rate of oxygen uptake was greater in treated than in untreated hypocotyls. Their rates of carbon dioxide release, however, were equal. Thereafter both rates were higher in treated than in untreated hypocotyls and continued to be so throughout the 7 days of the experiment (fig. 2).

The respiratory quotient of untreated hypocotyls was initially less than 1 but then rose rapidly to 1, where, within the limits of error, it remained. The respiratory quotient of treated hypocotyls continued to be less than 1 for 3 days, after which time it, too, became and remained about unity.

The percentage dry weight of untreated hypocotyls dropped rapidly to a minimum and then began to rise again. After a 2-day delay, treated hypocotyls followed an almost identical pattern.

Application of 3% IAA had little effect upon the amount of total nitrogen per unit dry weight of the treated portion (table 1). There is indication that the soluble nitrogen fraction was somewhat increased by the treatment concurrent with a corresponding decrease in the protein nitrogen fraction. Similar results for total nitrogen as affected by IAA treatment have been reported by STUART (15) and by MITCHELL and MARTIN (12) if their data are recalculated on the basis of nitrogen per unit dry weight. Because of the uniformity of nitrogen content per unit dry weight of both treated and untreated samples throughout the experiment, calculation of respiratory rates on the basis of nitrogen content would give results nearly paralleling those found using dry weight as the basis. Consequently, respiratory rates were calculated on the basis of dry weight only.

Primary responses to IAA undoubtedly are soon complicated by effects of secondary, tertiary, and even higher order. Consequently, the changes which occurred soon after application were selected for detailed study. To this end a survey of different concentrations

effective over various short time intervals was undertaken. Results (fig. 3) show that all concentrations used, even 10%, resulted in lowering the rate of fall of the respiratory rate, with the magnitude of response in the order of the concentration (see also table 4). Significant effects were noted after as short a period as 6 hours.

The rate of anaerobic respiration of treated hypocotyls 24 hours after application of 1% IAA was 115% of that of untreated hypocotyls (fig. 4). Ethyl alcohol formed accounted for 95% of the carbon dioxide released by slices from treated hypocotyls and for 92% in those from untreated hypocotyls. The ethyl alcohol analyses represent the average of duplicates from one experiment. Although percentages of carbon dioxide accounted for by the alcohol were not significantly different, the total amounts of alcohol formed were. As previously mentioned, the attempt to determine lactic acid was unsuccessful. In view of these results, IAA seemed to have little or no effect upon the end products of anaerobic respiration.

MITCHELL *et al.* (11) described the responses of slices from several plants and plant parts to treatment with IAA in aqueous solution. Except for bean roots, no increase in rate of respiration from the IAA treatment was found but rather, with higher concentration, greater inhibition. The results found for bean roots were explained by the presence there of an IAA-oxidizing enzyme. Since most of their work was done with tomato stem tissue and only the results of one concentration (2×10^{-3} *M*) were given for bean stem tissue, a brief survey of the effects of lower concentrations on bean hypocotyl tissue was made. Results (table 2) are inconclusive, since significant increases were observed in some instances; in others, not. Whatever effects IAA in aqueous solution may have on slices of the bean hypocotyl, they are not so marked as that of IAA in lanolin applied to the intact hypocotyl.

An attempt was then made to deter-

TABLE 1

NITROGEN CONTENT AND PERCENTAGE DRY WEIGHT OF MARKED ZONES OF GREEN BEAN PLANTS FOLLOWING UNILATERAL APPLICATION TO HYPOCOTYLS OF 3% IAA IN LANOLIN (U, UNTREATED; T, TREATED)

		Days after application							
		0	1	2	3	4	5	6	7
U.....	Total N*	70	68	68	68	65	66	63	57
	Sol. N*†	41	40	44	47	44	42	40	38
	Prot. N*	29	28	24	21	21	24	23	19
T.....	Total N*	.....	66	68	68	66	65	69	68
	Sol. N*†	.....	42	46	49	47	42	49	48
	Prot. N*	.....	24	22	19	19	23	20	20
U.....	% Dry wt.	9.9	7.1	6.1	5.6	6.0	6.1	6.4	6.8
T.....	% Dry wt.	.....	9.6	8.3	6.8	6.1	5.7	6.0	6.4

* Figures in mg. N/gm. dry weight.
† Not including nitrate N.

mine if IAA was acted upon by the intact hypocotyl in such a way as to form a more active substance (table 3). Juice pressed from untreated or treated green hypocotyls was added to slices from untreated green hypocotyls. Marked increases in rates of respiration occurred, but the increase induced by juice from treated hypocotyls was no greater than

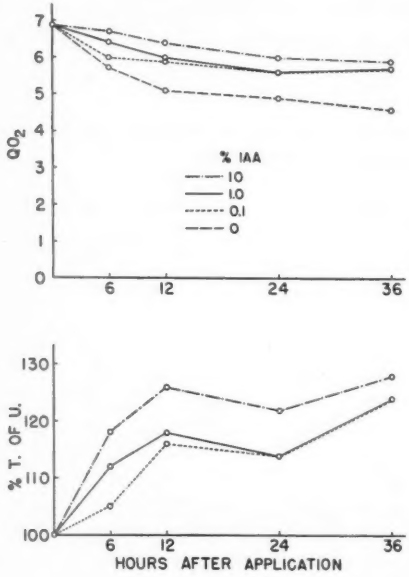

FIG. 3.—*Upper:* cu. mm. oxygen uptake per mg. dry weight per hr. (QO_2) of slices from green bean hypocotyls at various times following unilateral application of different concentrations of IAA in lanolin. *Lower:* as above, with oxygen uptake of slices from hypocotyls receiving IAA expressed as percentage of oxygen uptake of those receiving no IAA (% T of U).

that from untreated hypocotyls. When IAA was added with the juice from treated hypocotyls, there was no additional effect. The increase in rate was immediate but continued for only an hour or less, after which time the respiratory rate became equal to or lower than that of untreated slices. Juice kept in a boiling water bath for 5 minutes produced no effect upon respiration, and rapid loss of activity of the juice occurred with standing at room temperature. Since no effect of IAA could be established in these experiments, this part of the work was not carried further.

ETIOLATED PLANTS

A comparison of the effectiveness of the two methods previously described for introducing IAA into lanolin showed any particular IAA concentration in ether-mixed paste to be about ten times as effective as that IAA concentration in paste mixed by stirring only (table 4). Because of its effectiveness, 1%—unless otherwise specified—IAA mixed into lanolin by means of ether was used in all subsequent experiments.

An attempt was made to determine how soon after application of the paste a noticeable response could be detected. Unsliced pieces about 8 mm. long taken from immediately below the arch were treated in the usual way with either lanolin or IAA in lanolin and placed in vessels for measurement of oxygen uptake as described earlier. Results (fig. 5) show that the effect of IAA may be detected as early as 1 hour after application.

An intensive study was made of the effect of the 1% IAA treatment on growth and respiration of the marked zone (fig. 1, $B + C$) of the etiolated hypocotyl (table 5). Elongation was inhibited, with the greatest effect coming between 12 and 24 hours after application. The same time pattern was evident in dry-weight changes. However, in this case the rate of gain of dry weight was increased. The fresh weights and volumes were nearly identical and showed little effect of the treatment throughout the first 24 hours, though by 48 hours the

treated had surpassed the untreated. Similarly, there was no significant effect of treatment on the rate of water uptake until the second day, when the treated did take up more than the untreated. However, the percentage dry weight of treated tissue continued higher, though not proportionately so. The effects of treatment upon respiratory rate were of the kind reported previously in this paper and were correlated more with the dry-weight than with any other changes.

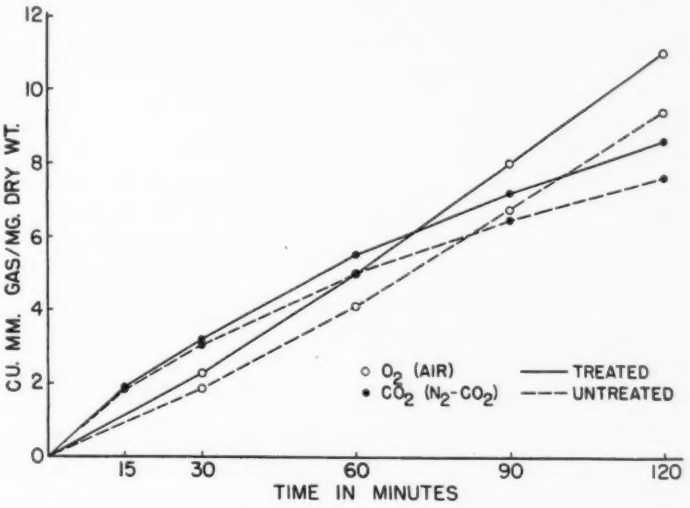

FIG. 4.—Cu. mm. of oxygen uptake and anaerobic carbon dioxide release per mg. dry weight [O_2 (air) and CO_2 (N_2–CO_2)] of slices from green bean hypocotyls 24 hours after unilateral application of 1% IAA in lanolin (treated), or untreated.

TABLE 2

EFFECT OF AQUEOUS SOLUTIONS OF IAA ON OXYGEN UPTAKE OF SLICES FROM UNTREATED BEAN HYPOCOTYLS
(OXYGEN UPTAKE IN ABSENCE OF IAA = 100)

KIND OF HYPOCOTYL	ADDED SUBSTRATE	pH	CONCENTRATION OF IAA (MOLARITY)				
			10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}
Green	.....	6	103	100	105	105	89
	.....	5	.....	.....	103	.....	58
	1% sucrose	6	98	107	109	114	.....
	1% sucrose	6	.....	.....	.....	111	.....
	.....	6	.....	.....	.....	113	.....
Etiolated	.....	6	.....	105	103	100	73
	.....	6	102	98	97	95	72

Although most marked effects of the unilateral application to the hypocotyl of IAA in lanolin occur locally, responses of the rest of the plant should not be overlooked. To follow these, plants were divided into the five parts previously described—epicotyl, cotyledons, upper hypocotyl, lower hypocotyl, and roots. Results are given in table 6.

LINK *et al.* (10) first reported the retardation of epicotyl development resulting from treatment of the intact hypocotyls of green and etiolated bean plants with an IAA-lanolin paste and

TABLE 3

EFFECTS OF HYPOCOTYL JUICE ON OXYGEN UPTAKE OF SLICES FROM GREEN HYPOCOTYLS (OXYGEN UPTAKE IN ABSENCE OF JUICE = 100)

PERCENTAGE JUICE IN SUBSTRATE	TYPE OF HYPOCOTYL SERVING AS SOURCE OF JUICE	FURTHER TREATMENT OF JUICE	OXYGEN UPTAKE	
			1st Hr.	2d Hr.
20	Untreated	.....	131	97
	Treated with 1% IAA	.....	124	107
	Treated with 1% IAA	10^{-5} *M* IAA added	122	108
	Treated with 1% IAA	No slices present	1	0
40	Untreated	.....	137	101
	Treated with 1% IAA	.....	144	101
	Untreated	.....	138	89
	Untreated	Pressed 1 hr. before 0 time	121	84
	Untreated	In boiling-water bath for 5 min.	96	103
	Untreated	.....	126	70

TABLE 4

EFFECTS OF TWO METHODS OF INTRODUCING IAA INTO LANOLIN ON OXYGEN UPTAKE AND RESPIRATORY QUOTIENTS OF SLICES FROM ETIOLATED BEAN HYPOCOTYLS TREATED WITH IAA IN LANOLIN

CONC. OF IAA IN LANOLIN (% BY WT.)	TIME AFTER APPLICATION (HR.)	METHOD OF PREPARATION OF IAA-LANOLIN PASTE				
		Stirring		Ether and stirring		
		QO_2*	%T of U†	QO_2*	%T of U†	RQ‡
0.0	0	6.5	100	6.4	100	0.81
	24	4.6	100	4.5	100	0.90
0.001	24	5.0	109	5.1	113	0.85
0.01	24	5.2	113	5.3	118	0.82
0.1	24	5.2	113	5.9	131	0.78
1.0	24	5.9	128	6.8	151	0.79

* Cu. mm. O_2 uptake/mg. dry weight/hr.
† % treated of untreated.
‡ Respiratory quotient.

mentioned similar effects from treatment of first internodes of etiolated plants. The effects of treatment of the intact hypocotyls of green plants were more completely investigated by LINK and KLEIN (9); the effects of treatment of the first internodes of etiolated beans, by MITCHELL and MARTIN (12). LINK *et al.* (10) also reported retardation of elongation of the first internodes of green bean plants after treatment of the first internodes; HAMNER and KRAUS (6) reported no effect on stem elongation of similar treatment of the second internodes of green bean plants. In the course of the studies here reported, the writer also observed but did not measure the initial inhibition of growth and development of epicotyls of green plants after application of IAA in lanolin to the intact hypocotyls. Data on the inhibition of growth and respiration of the epicotyl resulting from similar treatment of etiolated plants are recorded in table 6. The rate of growth was greatly reduced during the first 24 hours following application; after that time, however, the rate nearly returned to normal. Rate of respiration was affected similarly, being greatly inhibited at the end of 12 hours but after 24 hours again approaching the rate of respiration of epicotyls of untreated plants.

MITCHELL and MARTIN (12) found that 4 days after IAA application to the first internodes of etiolated plants the cotyledons of treated plants had lost significantly less weight than those of untreated plants. The results of hypocotyl treatment (table 6) show only a tendency in this direction by the end of 48 hours, with no difference until then. Hypocotyl treatment induced no significant effect upon the rate of respiration of the cotyledons.

Treatment of the etiolated hypocotyl directly below the arch with 1% IAA in lanolin induced negative curvature of the upper hypocotyl; this resulted in an increase in the curvature of the arch by approximately 90° at the end of 12 hours. The arch then straightened but not so rapidly as in untreated seedlings (fig. 1). Rate of growth of the upper hypocotyl followed the same general pattern of inhibition by IAA treatment as

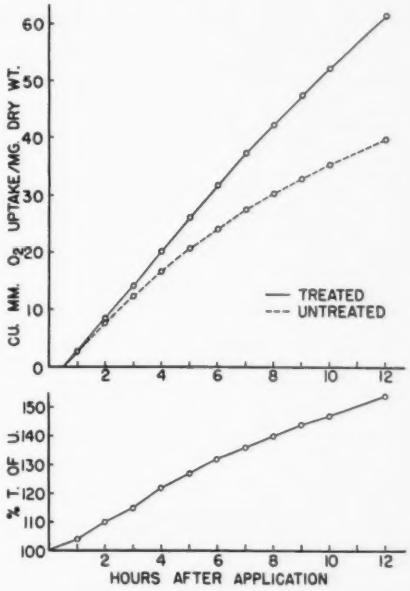

FIG. 5.—*Upper:* cu. mm. of oxygen uptake per mg. dry weight of unsliced pieces of etiolated bean hypocotyls following unilateral application of lanolin alone (untreated) or 1% IAA in lanolin (treated). *Lower:* as above, with oxygen uptake of treated pieces expressed as percentage of that of untreated (% T of U).

did that of the epicotyl; however, inhibition of the respiratory rate of the upper hypocotyl was not comparable to that of the epicotyl. After 12 hours the respiratory rate of upper hypocotyls of treated plants was only slightly inhibited; after 24 hours it appeared to be higher than that of untreated plants. Because of the

rapid elongation of the upper hypocotyls of untreated plants (fig. 1), it seemed that partial explanation might lie in the rapid fall in respiratory rate of the maturing lower parts of the upper hypocotyls (fig. 1, *F*). This proved to be the case (table 7).

Allowing for inclusion of the entire lower portion of the treated part of the hypocotyl and for experimental variation, the growth and respiratory responses of the entire lower hypocotyl (fig. 1, *A*) are the same as those of the marked zone (fig. 1, *B* + *C*).

Roots showed no response of any kind to hypocotyl treatment.

Although responses of certain individual parts to application were marked, responses of the plant as a whole were much less so. The treated plant was shorter, its development was retarded, and its rate of increase in fresh weight reduced slightly; however, no significant difference in dry weight was noted. Loss in dry weight of etiolated plants grown in sand without nutrient solution can be used as a measure of respiratory rate. On this basis, treatment with IAA did not affect total respiration of the plant. Using dry-weight and QO_2 averages, the oxygen uptake of the plant per hour was calculated. This method also showed no

TABLE 5

EFFECT ON GROWTH AND RESPIRATION OF MARKED ZONES OF ETIOLATED BEAN HYPOCOTYLS FOLLOWING UNILATERAL APPLICATION OF 1% IAA IN LANOLIN

(DATA ON A PER PLANT BASIS. U, UNTREATED; T, TREATED)

TIME AFTER APPLICATION (HR.)	U	T	%T OF U	U	T	%T OF U
	Fresh wt. (mg.)			Length (mm.)		
0	207			16.2		
12	377	372	99	27.0	23.2	86
24	540	545	101	38.6	24.6	64
48	616	724	117	44.4	27.6	62
	Dry wt. (mg.)			Volume (cu. mm.)		
0	12.6			210		
12	19.0	20.1	106	381	380	100
24	23.0	30.9	134	567	562	99
48	27.7	37.9	137	647	738	114
	% Dry wt.			QO_2* (cu mm/mg dry wt/hr)		
0	6.1			6.0		
12	5.9	5.4	108	5.3	6.3	119
24	4.3	5.7	132	4.3	6.1	142
48	4.5	5.2	116			

* Based on slices from upper zone (fig. 1, *B*).

significant differences resulting from treatment. MITCHELL and MARTIN (12), using dry-weight changes and carbon dioxide production by the entire plant, obtained similar results with etiolated plants treated at the first internodes.

Respiratory measurements of both slices and unsliced pieces were made for hypocotyl and epicotyl. Comparison of untreated and treated hypocotyl tissue indicates that the two methods give essentially the same results (table 7), although rates of respiration for unsliced pieces were slightly lower than for the corresponding slices. The unsliced pieces showed somewhat greater inhibition of the respiration of the epicotyl by treatment than did the slices (table 7), al-

TABLE 6

EFFECT ON GROWTH AND RESPIRATION OF ENTIRE ETIOLATED BEAN PLANTS FOLLOWING UNILATERAL APPLICATION OF 1% IAA IN LANOLIN TO HYPOCOTYLS
(DATA ON A PER PLANT BASIS. U, UNTREATED; T, TREATED)

Part of plant	Time after application (hr.)	Growth (mg.)				% dry wt.		Rate of growth* (mg/gm/hr)				Length (mm.)		Rate of oxygen uptake†			
		Fresh wt.		Dry wt.				Fresh wt.		Dry wt.				QO_2 (cu mm/mg dry wt/hr)		Cu. mm/part of plant/hr‡	
		U	T	U	T	U	T	U	T	U	T	U	T	U	T	U	T
Epicotyl	0	89		14.0		15.7								8.7		122	
	12	140	109	22.2	17.8	15.9	16.3	37	17	38	12			7.7	5.9	171	105
	24	184	123	28.7	20.0	15.6	16.3	23	10	21	10			6.1	5.6	175	112
	48	330	228	45.1	33.4	13.7	14.6	24	25	19	21						
Cotyledons	0	851		282.9		33.3								1.0		283	
	12	793	755	239.1	236.2	30.2	31.3	− 6	−10	−14	−14			1.1	1.2	263	283
	24	695	660	197.3	195.2	28.4	29.6	−11	−11	−16	−16			1.4	1.3	276	254
	48	482	488	119.0	135.8	24.7	27.8	−15	−12	−21	−15						
Upper hypocotyl	0	134		12.7		9.5						22		5.4		69	
	12	236	163	19.0	17.4	8.1	10.7	46	16	33	26	31	26	4.3	4.1	82	71
	24	514	243	30.0	22.0	5.8	9.1	62	33	38	19	53	30	3.9	4.0	117	88
	48	1340	998	57.7	45.7	4.3	4.6	37	51	26	29	125	57				
Lower hypocotyl	0	632		31.8		5.0						45		4.0		127	
	12	867	880	41.5	46.8	4.8	5.3	26	27	22	32	61	56	2.9	4.0	120	188
	24	938	1106	43.0	62.4	4.6	5.6	7	19	3	24	74	58	2.6	3.3	112	206
	48	1059	1256	47.0	63.2	4.4	5.0	5	11	4	1	81	62				
Roots	0	299		18.9		6.3								7.2		136	
	12	386	359	22.8	23.0	5.9	6.4	21	15	16	16			7.0	7.0	160	161
	24	450	469	23.8	25.3	5.3	5.4	13	22	4	8			6.2	6.3	148	160
	48	544	606	27.0	26.4	5.0	4.4	8	11	5	2						
Total	0	2005		360.3		18.0								2.0		737	
	12	2422	2266	344.6	341.2	14.2	15.0	12	10	− 4	− 5			2.3	2.4	796	808
	24	2781	2610	322.8	324.9	11.6	12.5	11	12	− 5	− 4			2.6	2.5	828	820
	48	3755	3576	295.8	304.5	7.9	8.5	12	13	− 4	− 3						

* Based on the average rate per period—0–12, 12–24, 24–48 hours.
† Results obtained with unsliced pieces.
‡ Calculated by multiplying the QO_2 by the dry weight of the plant part.

though both methods seemed to give rates of respiration of the same magnitude.

Treatment with 0.001% IAA in lanolin had little effect on growth, though slightly higher fresh and dry weights of the marked zones (fig. 1, $B + C$) were found rather consistently in treated plants (table 8); nevertheless, this low concentration did cause definite changes in the respiration of the marked zones (table 4).

That the responses observed resulted from diffusion of IAA from the lanolin into the hypocotyl can hardly be questioned; however, just how much enters, to what parts it is distributed, and its final disposition are still unknown. Such information would help in interpretation of the above results. To obtain an indication of the amount of IAA which had diffused into the hypocotyl the ferric chloride colorimetric test as modified by TANG and BONNER (16) was applied to aqueous sodium bicarbonate fractions of ether extract concentrates; but all trials were unsuccessful even in obtaining a color response which could be attributed definitely to IAA and not to interfering substances in the concentrates.

Discussion

Any given part of the untreated hypocotyl has an initial high rate of respiration which decreases rapidly as the hypocotyl grows and matures. This is true whether the respiratory rate is calculated on a dry-weight or on a nitrogen basis. In a few instances application of IAA was followed by an actual, though slight, increase in respiratory rate (tables 4, 5; fig. 2). In most instances, however, the respiratory rate of treated tissue continued at about the same rate or dropped only slightly (tables 4, 6; figs. 3, 5). The effect of IAA, therefore, seems more an inhibition of the normal decline than an actual increase in the respiratory rate.

The respiratory responses of green hypocotyls to treatment with IAA seem well correlated with the morphological changes reported by LINK and co-workers (9, 10). Their findings indicated inhibition of maturation of the tissue at the site of treatment—also reported for the *Avena* coleoptile by WENT (21)—with more or less isodiametric increase in cell size, stimulation of further cell division, and eventual formation of adventitious root primordia. This inhibition of maturation was also indicated by prolongation of the initial high respiratory rate, continuation of a low respiratory quotient, and decreased rate of fall of the percentage of dry weight.

Comparison of aerobic with anaerobic respiration of green hypocotyl tissue and the effect thereon of treatment with IAA is somewhat difficult because of changes in the rates of gas exchange with time (fig. 4). The rate of oxygen uptake is steady to slightly increasing, whereas the rate of anaerobic carbon dioxide release falls off quite rapidly. Another striking difference is that the treated-untreated ratio for rates of oxygen uptake is higher than 1 and constant; however, the treated-untreated ratio for the rates of anaerobic carbon dioxide release is initially 1, but becomes increasingly greater than 1 with time during the 2-hour experimentation period. The significance of this is not clear.

Not enough work was done to allow definite conclusions on the effect of IAA treatment upon the rate of glycolysis; however, the lag in the normal rise of the respiratory quotient is indicative of some difference in the balance of carbohydrate, protein, fat, and organic acid oxidation, with possibly smaller than normal usage of the glycolytic pathway

of carbohydrate catabolism. The fact that more alcohol was formed by the treated than by the untreated tissue during a 2-hour period of anaerobic respiration does not preclude an increased activity of alcohol dehydrogenase as found by BERGER and AVERY (4) in *Avena* coleoptiles previously treated with IAA.

IAA treatment of etiolated hypocotyls seemed to inhibit maturation of the tissue as described for green hypocotyls.

TABLE 7

EFFECT ON RESPIRATION OF PARTS OF ETIOLATED BEAN PLANTS FOLLOWING UNILATERAL APPLICATION OF 1% IAA IN LANOLIN TO HYPOCOTYLS

MEASURED FOR SLICES AND UNSLICED PIECES
(U, UNTREATED; T, TREATED)

PART OF PLANT	TIME AFTER APPLICATION (HR.)	UNSLICED PIECES			SLICES		
		QO_2*		%T of U	QO_2*		%T of U
		U	T		U	T	
Epicotyl	12	7.7	5.9	77	7.0	6.4	91
	24	6.1	5.6	92	5.3	6.1	115
Upper hypocotyl	12	4.3	4.1	95	6.0	5.9	98
	24	4.0†	4.0	100	5.7†	5.5	96
	24	3.8‡		105	4.6‡		120
Marked zone of lower hypocotyl	12	4.0	5.4	135	4.8	6.1	127
	24	3.2	4.3	134	3.8	5.6	147

* Cu. mm. O_2 uptake/mg. dry weight/hr.
† Upper half of upper hypocotyl (fig. 1, *E*).
‡ Lower half of upper hypocotyl (fig. 1, *F*).

TABLE 8

GROWTH OF ETIOLATED HYPOCOTYLS FOLLOWING UNILATERAL APPLICATION OF 0.001% IAA IN LANOLIN
(DATA ON A PER PLANT BASIS. U, UNTREATED; T, TREATED)

PART OF PLANT	TIME AFTER APPLICATION (HR.)	FRESH WT. (MG.)		DRY WT. (MG.)		LENGTH (MM.)	
		U	T	U	T	U	T
Upper hypocotyl	0	116	.....	11	.....	20	.....
	12	206	195	16	17	30	29
	24	458	435	27	26	50	47
	48	1279	1282	48	51	117	114
Marked zone of lower hypocotyl	0	204	.....	13	.....	16	.....
	12	360	408	18	20	26	28
	24	544	537	22	24	38	37
	48	632	727	28	33	46	44

The correlation of respiratory rate with changes in dry weight rather than with those of volume and water uptake indicates that the high rate of respiration is involved in the accumulation of building material and the synthesis of protoplasm leading to cell division and proliferation of tissue rather than to cell enlargement resulting primarily from water uptake. In fact, the rate of respiration of untreated tissue falls rapidly during its period of rapid elongation, although at this time it is enlarging as rapidly as treated tissue. Throughout this period both are taking up about the same amount of water; but because of the difference in dry-weight increase and in direction of cell expansion, the growth is quite dissimilar.

There are certain interesting similarities and differences between results reported here after treatment of hypocotyls of etiolated beans and those reported by MITCHELL and MARTIN (12) after treatment of first internodes of etiolated beans. In both instances an increase in weight of only the hypocotyls was incited by treatment, whereas the increase in weight of epicotyls of treated plants was inhibited. The interesting difference is the site of treatment. Application of IAA to the first internode seemed to prevent transport of materials from the cotyledons and their accumulation on the far side of the application site; just the reverse was true in the hypocotyl application. These findings may indicate a disposition of various plant parts to react in a particular way to excessively high concentrations of IAA regardless of location of the source. Minor differences could then be explained by differences in age of tissue and in concentration of IAA—traceable to amount applied and site of application.

Explanations of the effects of IAA treatment on plant parts above the site of application must account for both the initial inhibition and the recovery to apparently normal growth. Two types of explanation might be offered for these responses.

It is possible that an increased gradient of building materials is set up by the hypocotyl, which enables it to draw more than its normal share of material from the cotyledons, which are unable to increase the rate of supply; consequently the inhibition assumes the nature of starvation. Somewhat similar hypotheses have been proposed (17, 22). Very rapid growth of the treated hypocotyl continues but a short time after which more building material from the cotyledons is again available to the inhibited parts, and they then resume an essentially normal growth rate (table 6). This explanation assumes little movement of the applied IAA in an upward direction.

It is also possible that some IAA diffuses into the xylem and is translocated rapidly upward, where it acts to inhibit growth and respiration of the epicotyl similarly to bud inhibition. THIMANN (17) has discussed the possible mechanisms of bud inhibition. It would then be necessary to account for the disappearance of such high concentrations of IAA, which must precede any return to growth and respiratory rates similar to those of epicotyls of untreated plants. Such could occur through a decrease in upward transport of IAA because of destruction in the hypocotyl or divergence elsewhere and/or increasing its destruction in the epicotyl with or without retranslocation downward. Acquired immunity to abnormally high IAA content hardly seems plausible.

Since no direct evidence concerning this situation was available, previous

reports were re-examined for indications of the probable courses and dispositions of IAA applied in lanolin to the bean hypocotyl. Many data have been accumulated, and many explanations have been proposed that bear on this problem. Since this paper is not a review (see **1, 5, 17**), only references to work with bean will be cited directly.

REDEMANN *et al.* (**14**) reported the presence of oxidizing substances in unpurified U.S.P. anhydrous lanolin—the type used throughout these experiments—capable of inactivating IAA. This factor was of probable importance in the lowest concentration used, 0.001%. They also reported the appearance of negative curvature of bean stems with that concentration in purified lanolin whereas, as reported in this paper too, no growth response was obtained at the same concentration in unpurified lanolin. An effect upon respiration, however, following application of 0.001% IAA is reported in this paper. It is doubtful that at the higher levels, especially the 3% or 1% used most extensively, any appreciable amount of the IAA present in the lanolin was destroyed by such oxidizing substances.

The amounts of IAA applied were undoubtedly much higher than those effective in the untreated plants; and, on the basis of the findings of VAN OVERBEEK and co-workers (**19**) for etiolated bean hypocotyls, of the order of ten to several thousand times the amount normally contained in a hypocotyl. The colorimetric work with the concentrated extracts, previously mentioned, indicated only that relatively little of the amount applied entered or was not destroyed by the hypocotyl. However, little of the IAA applied would be needed to increase by several fold the small amount probably present in the untreated hypocotyl. This problem will remain an open question until some method is devised for accurately determining the IAA effective in the normal plant.

The occurrence of enzymes capable of destroying IAA is well established. LARSEN (**8**) reported such in the etiolated epicotyls of *Phaseolus multiflorus;* and WAGENKNECHT and BURRIS (**20**) in the roots of *Phaseolus vulgaris* var. Yellow Wax, though they were unable to demonstrate its presence in the green parts of bean plants. This makes probable the destruction of at least some and perhaps most of the entering IAA, especially in etiolated hypocotyls, which does not lessen the uncertainty of how much of the added IAA might have been effective.

That any IAA destruction is oxidative in nature is also probable; however, it is generally considered that the indole ring structure is left intact. If such is the case, oxidation of the entire amount of about 100 μgm. of IAA applied to a bean hypocotyl in 1% IAA-lanolin paste would require only about 13 cu. mm. of oxygen. Considering that the oxygen requirement of the lower hypocotyl at the time of application is about 127 cu. mm. per hour (table 6), it does not seem possible that any appreciable portion of the respiratory effect can be traced to oxidation of the applied IAA. This is further indicated by the results of addition of IAA in aqueous solution to slices (table 2) and to slices and juice (table 3).

LINK *et al.* (**10**) pointed out that the cutinized epidermis of green hypocotyls may prevent the entrance of much of the applied IAA. The difference in epidermal permeability might be the primary reason that etiolated hypocotyls react more quickly and intensely than green ones.

The courses and dispositions of IAA

which does diffuse into and is not destroyed by the hypocotyl are also uncertain. Using applications of 0.5% IAA in lanolin, BEAL (3) found evidence that movement of IAA through the second internode of the bean takes place almost entirely in a basipetal direction with little, if any, acropetal movement. JACOBS (7) reported that at a concentration of 2 p.p.m. IAA is transported only basipetally through 5-mm. sections taken from the upper parts of bean hypocotyls. Work with other plants, however, indicates and it is generally accepted that, when applied in high concentration, IAA may leak into the xylem and be transported upward rapidly with the transpiration stream (1, 5, 17). The slower diffusion upward through other tissues from a high concentration cannot be entirely dismissed.

LINK *et al.* (10) interpreted the occurrence of swellings above, below, and lateral to the site of application of IAA in lanolin as evidence of the movement of IAA upward, downward, and transversely in the bean plant. The position and developmental shape, including a rather sharp upper boundary, of the swelling resulting from the application of IAA to both green and etiolated hypocotyls as observed throughout the work reported in this paper indicates that initially most of the effective IAA moves in a lateral and downward direction while the later disappearance of the sharp upper boundary of the initial swelling by an increased swelling above the site of application may indicate movement of IAA upward. It may, however, merely indicate that cells dividing in the zone reached by the introduced IAA affect their neighbors in some way to initiate cell enlargement and division. This may be mediated through changed growth substance relations in the cells. This does not preclude possibility of an earlier movement upward of IAA which might have diffused into the xylem. In fact, as mentioned earlier, such movement might account for the inhibitory effects on growth and respiration of the treatment upon the upper hypocotyl and epicotyl.

The above indicates two possibilities: (*a*) Most of the IAA diffusing into the hypocotyl is destroyed so that the concentration remains low enough to prevent nonpolar movement upward; however, the continuing supply of IAA from the applied paste maintains a sufficiently high concentration in the hypocotyl to account for the effects at, below, and slightly above the treatment site. This explanation favors the "starvation" hypothesis given earlier. (*b*) It may be, however, that relatively little IAA is destroyed by the hypocotyl and that it is in sufficient concentration to be translocated upward. This line of reasoning favors the bud-inhibition hypothesis.

Summary

1. Study was made of the effects upon respiration and growth following unilateral application of IAA in lanolin on or directly below the arch (on the convex side) of young seedlings of *Phaseolus vulgaris* var. Red Kidney. Both green and etiolated plants were used.

2. IAA incorporated into lanolin with ether was almost ten times as effective in eliciting response as that mixed into lanolin by stirring only.

3. Slices and unsliced pieces of hypocotyl and epicotyl gave comparable results in the measurement of oxygen uptake.

4. The rate of respiration of untreated hypocotyls, though initially high, fell rapidly with time; however, treatment with IAA prolonged the high initial rate

of respiration. The respiratory quotient of untreated hypocotyls, though initially below 1, rose rapidly to 1; however, treatment with IAA delayed this rise. These results corroborate the previously reported histological evidence of a delay in maturation resulting from IAA treatment.

5. The rate of anaerobic respiration of treated hypocotyls was higher than that of untreated; however, the ethyl alcohol produced accounted for 92%–95% of the carbon dioxide released by both treated and untreated hypocotyls.

6. Respiration and growth of epicotyl and upper hypocotyl were initially inhibited by treatment. Later these returned to about the same rates found in untreated parts of the same age. Two possible explanations of this response are proposed.

7. The effects of treatment upon the lower hypocotyl were inhibition of elongation; more rapid increase in diameter, volume, dry weight, and fresh weight; and reduction in the rate of fall of the respiratory rate. The high rate of respiration of the treated lower hypocotyl was correlated more nearly with the rapid dry-weight increase than with the slower fresh-weight or volume increases.

8. Oxidation of IAA in the hypocotyl cannot account for the effects upon respiration.

9. The effect of treatment upon the respiration of the hypocotyl was detectable within 1 hour.

10. Treatment produced no significant effects on either the cotyledons or the roots.

11. The increased rate of respiration of some parts of the treated plants was counterbalanced by decreases in other parts with the result that the respiration of the plant considered as a whole was not affected by treatment with IAA.

12. Though producing an effect upon the rate of respiration, 0.001% IAA had little effect upon growth.

13. A concentration of 10^{-3} *M* IAA in water inhibited the respiration of hypocotyl slices. Lower concentrations produced no significant effects.

14. Juice from untreated and treated hypocotyls added as substrate to hypocotyl slices produced the same effects of initial increase in the rate of respiration followed by a decrease to or below the rate for untreated slices.

15. Similarities and differences in the responses of etiolated plants to treatment of the first internodes and of the hypocotyls are noted and discussed.

16. The possible courses and ultimate dispositions of IAA applied in lanolin paste to the intact hypocotyl are discussed.

The writer wishes to express his appreciation to Professor G. K. K. Link for his encouragement and counsel during the course of this work.

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FRUIT MORPHOLOGY AND ANATOMY OF THE CHERIMOYA

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Introduction

The development of the cherimoya (*Annona cherimola* Mill.) as a fruit industry of potential, though at present minor, importance in California has presented many botanical and horticultural problems concerning which little is known at present. Information on the anatomy and morphology of this fruit is especially scant and scattered in the literature. The present study was begun to ascertain some of the basic facts concerning the structure and general morphology of the fruit.

The most extensive investigation on the histology of annonaceous fruits in general is that by LICOPOLI (**4**). He described the morphology and gross anatomy of *Annona reticulata* L. (Bullock's heart) and of the close relative *Asimina triloba* (L.) Dunal (American papaw). JULIANO (**2**) followed the development of the megagametophyte of *A. squamosa* L. but did not consider fruit development beyond the mature embryo-sac stage. NICOLASI-RONCONTI (**5, 6**) investigated development of the gametophyte and seed of *A. cherimolia* but reported no

other information concerning anatomy of the fruit. SMITH (9), in a general comparative survey of the vascular anatomy of primitive families, including the Annonaceae, confined his studies to the early floral stages where he considered only the vascular systems.

VOIGT (10) made a comprehensive study of seed development in several families including Annonaceae. He showed among other things that the ruminate endosperm in this family resulted primarily from invagination by the inner integument. WINTON and WINTON (11) compiled from several sources very meager and brief descriptions and illustrations of several annonaceous fruits including the cherimoya but contributed little original knowledge on the fruit. A survey of seed anatomy in the family Annonaceae by CORNER (1) did not include the species *A. cherimola* but did cover the detailed anatomy of several close relatives.

Material and methods

The materials used consisted primarily of fresh fruit specimens of several horticultural varieties and seedlings of the cherimoya grown in the orchard of the Division of Subtropical Horticulture, University of California, Los Angeles. Mature fruits were sectioned free hand or with the aid of a sliding microtome. Some materials were killed and fixed in formalin–acetic-alcohol solution, imbedded in paraffin, sectioned, and stained with safranin and fast green or with Harris' haematoxylon. Drawings and measurements were made with the aid of a camera lucida. The ordinary reagents were employed to test for various ergastic substances and chemical constituents of the tissues.

Observations

GENERAL MORPHOLOGY

The fruit of the cherimoya is a syncarpium formed by fusion of several simple carpels into a solid mass. The individual carpels are arranged spirally on the surface of an elongated and tapered receptacle which is soft and pithy when the fruit is mature. Each carpel, of which there are between one and two hundred per fruit, contains a single ovule, although infrequently two ovules develop within a single carpel. A considerable percentage (50%–60%) of ovules generally do not develop because of inadequate pollination. Thus, cherimoya fruits are often distorted or asymmetrical because the carpellary wall increases in thickness only in those carpels which set seed. Fruit form generally is irregular and fruit-set poor and erratic unless adequate pollination is provided (8).

FORM.—Fruit form in the cherimoya is, in general, conical (fig. 1*A*). Considerable range is found, however, from almost spherical to long conical or nearly cylindrical (fig. 1*G–K*). Fruits of some varieties tend to have shoulders (very broad base with narrow apex), while others are more regular in outline. Variation in shape frequently exists even among fruits from a given tree. When adequate pollination has been provided, however, most of the horticultural varieties can be identified with a fair degree of certainty by their fruit shape as well as by surface characters.

SURFACE CHARACTER.—Perhaps the most useful character for identification and description of the cherimoya fruit is the nature of the fruit surface. These fruits have been classified horticulturally into five general types with respect to this character (7): These types are mammillate (*mammillata*), tuberculate (*tuber-*

culata), umbonate (*umbonata*), fingerprinted (*impressa*), and smooth (*laevis*) (fig. 1*B–F*). This classification is based on the nature of the individual areole or exposed surface of the carpel. Thus, "fingerprinted" fruits (fig. 1*E*) appear to have been pressed with the fingertip, each of the areoles having a slightly concave, smooth surface. Smooth fruits (fig. 1*F*) are lacking indentations, protuberances, or markings of any nature; the areoles are merely delineated by a dark line. Tuberculate and mammillate types (figs. 1*C*, *B*) are quite similar and differ primarily in that the latter has longer and more gently tapered protuberances. Fruits of the umbonate types have generally a small, sharp protuberance arising from a depressed areolar surface (fig. 1*D*). Complete intergradations among the surface types are often found on a single fruit. Fruit of a given horticultural variety and the individual fruit on a tree, however, are generally characterized by one of the above types of surface characters.

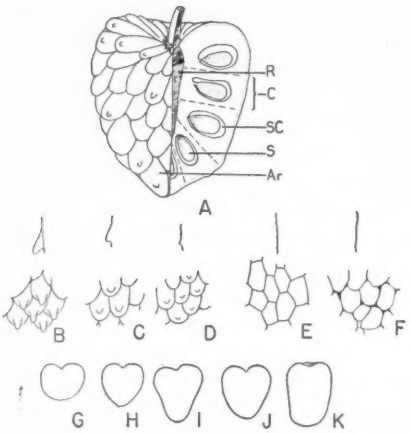

FIG. 1.—*A*, cherimoya fruit, surface and partial section view. *B–F*, surface characters: *B*, mammillate; *C*, tuberculate; *D*, umbonate; *E*, impressa; *F*, laevis. *G–K*, fruit forms: *G*, hemispherical; *H*, cordate; *I*, long conical, shouldered; *J*, short conical; *K*, oblong. *R*, receptacle; *C*, carpel; *S*, seed; *Ar*, areole; *SC*, seed cavity.

Each areole or fruit segment is delimited by a faint or dark line or in some cases by a slight ridge or furrow, or a combination of the two, which indicates the point of junction of adjacent carpels. Areoles near the fruit base are generally more irregular in outline than those near the apex. The outline of a well-developed areole is, in general, like an inverted U.

SIZE OF FRUIT.—The size attained by a fruit is almost directly proportional to the number of seeds which develop (8). Well-developed fruits of the Whaley variety, under California conditions, measure about 4–5 inches in diameter and weigh from 1 to $1\frac{3}{4}$ pounds. Under extremely favorable conditions, fruits almost twice this size are sometimes produced.

COLOR.—The fruit surface varies from very light to dark green, with some horticultural varieties having a definite yellow cast or russetting when mature.

FRUIT ANATOMY

OUTER EPIDERMIS.—The entire fruit surface is covered by soft epidermal hairs. These are two or three cells in length (150–601 μ long by 18–22 μ in diameter) and are gently tapered. The cell walls are of cellulose. Density of epidermal hairs varies among varieties. Very pubescent varieties such as Mira Vista have as many as 115–120 hairs per square millimeter, while at the other extreme some varieties such as Bays have only 25–68 hairs per square millimeter.

Stomata surrounded by simple guard cells (fig. 2*F*) are interspersed among the epidermal hairs over the entire fruit surface. No definite orientation of the stomatal openings is found. Stomatal density, however, varies from point to point on different segments of the fruit and is

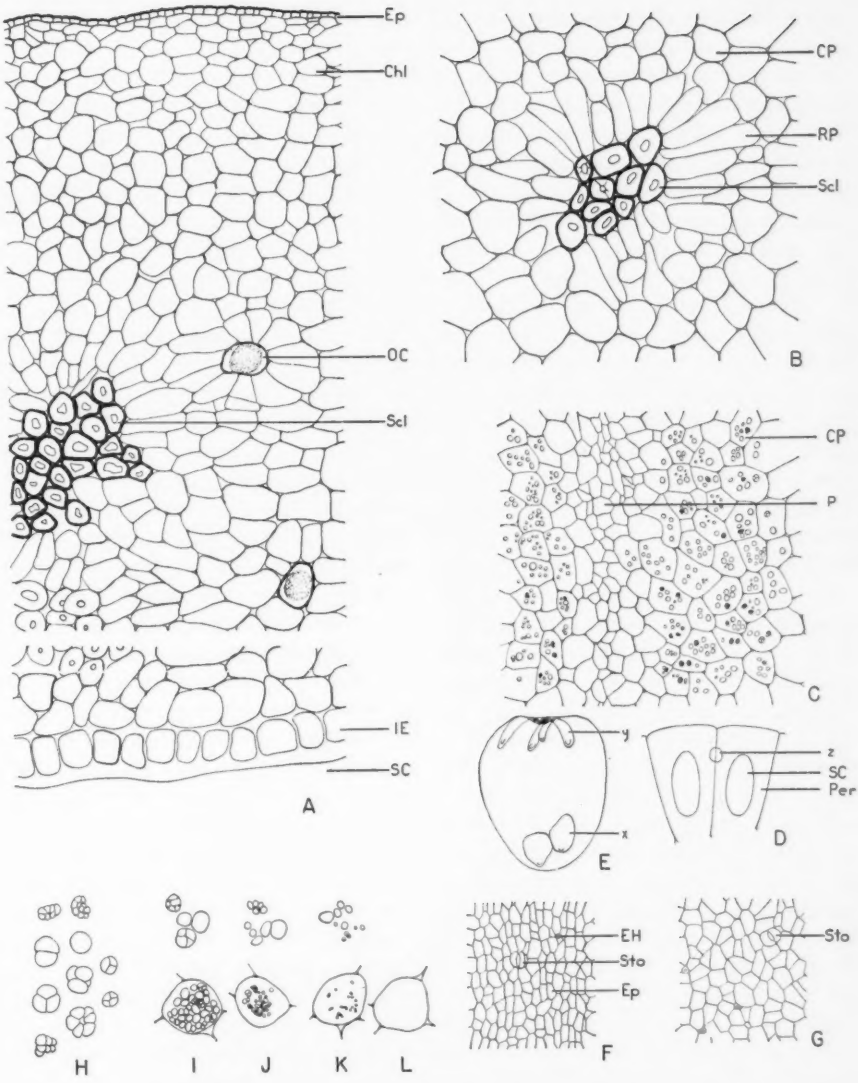

FIG. 2.—A, transverse section through pericarp wall. *B*, sclereid mass with "radiating" parenchyma. *C*, section through point *Z* of *D* showing line of union between adjacent carpels. *D*, two adjacent carpels in transverse section. *E*, diagram of "well" (*x*) and "poorly" (*y*) developed areoles. *F*, epidermis of areole *y*. *G*, epidermis of areole *x*. *H–L*, starch forms in parenchyma of firm and softening fruits. *Ep*, outer epidermis; *Chl*, chlorenchyma; *OC*, oil cell; *Scl*, sclerenchyma; *IE*, inner epidermis; *SC*, seed cavity; *CP*, cortical parenchyma; *RP*, "radiating" parenchyma; *Per*, pericarp wall; *EH*, epidermal hair; *Sto*, stomatal apparatus.

somewhat inversely proportional to the relative development of the areoles. Thus the areoles near the fruit apex which are better developed contain 5–23 stomata per square millimeter, whereas on the lesser developed areoles near the base of the same fruit the density is 40–46 per square millimeter. An individual stomatal opening measures 30 μ in length by 22 μ in width when fully open.

The outer epidermis is composed of simple cells rectangular in radial section and of variable outline in tangential section (fig. 2*F*, *G*). A cuticle, about 4 μ in thickness, and either smooth or slightly pebbled, covers the outer tangential surface.

MESOCARP.—Immediately beneath the epidermis is a layer of chlorenchyma ten to fifteen cells in depth. Beneath the chlorenchyma and extending partially into it is a layer of sclerenchyma about 4 mm. in thickness (fig. 2*A*). The latter consists of masses of thick-walled stone cells which are highly lignified. A typical sclereid is roughly isodiametric and measures 45–60 μ in cross section. Its cell wall is 12–15 μ thick, highly lamellated, and contains simple pits. The sclereids are lignified throughout but stain more intensely along the middle lamella with phloroglucin and HCl, becoming fainter toward the lumen; they are not affected by concentrated H_2SO_4 and IKI. These sclereids occur singly or in groups of two or twenty or more. The density and extent of development of the sclerenchyma are associated somewhat with skin or rind texture of the particular horticultural variety. Varieties which have relatively thin or fragile skins—for example, Bays—have small masses of sclereids separated by considerable parenchyma, while varieties with tougher rinds—such as Ryerson—have larger masses of sclereids with relatively less parenchyma.

There is surrounding each sclerenchymatous mass a layer of modified parenchymatous cells which, though somewhat irregular in shape, are in general elongated with their main axes radiating in all directions from the mass of sclereids (fig. 2*B*). The walls of the "radiating parenchyma" are somewhat thicker than those of adjacent parenchyma, and a relatively greater abundance of tannin materials is found in these cells.

The general mass of the carpellary wall which forms the major portion of the fruit consists of parenchyma. The parenchyma of any given carpel is separated from adjacent carpellary wall tissue by a layer of small parenchymatous cells, also very thin walled and with little ergastic material, which extends radially from the receptacle to the fruit surface (fig. 2*C*). Near the surface of the fruit, however, this tissue merges completely with the regular parenchyma.

INNER EPIDERMIS.—An epidermal layer borders the seed cavity. Its cells, rectangular in cross section and elongate and somewhat pointed in tangential section, range in size from 15 to 20 μ wide by 100 to 185 μ long. The tangential wall next to the cavity is covered by an uncutinized membrane about 7 μ thick. The radial walls are also thickened (1.5–2.0 μ thick). This layer of cells is conspicuous in that the separation of adjacent cells occurs irregularly with the formation of intercellular spaces along the radial walls. These internal epidermal cells also are strikingly devoid of ergastic materials, though occasionally a few small starch grains are found.

VASCULAR ANATOMY.—The vascular supply to the fruit enters through the fruit stem as a collateral dictyostele. Considerable sclerenchyma is found in the cortex of the fruit stem, and small groups of sclerenchymatous cells are located in its pith.

The vascular cylinder is an undissected ring of xylem as it enters the receptacle (fig. 3*B*, *C*). Within the receptacle it branches profusely into a peripheral network of several main bundles which divide and anastomose. This reticulate cylinder of xylem tissue extends through the entire length of the receptacle and is progressively reduced in amount toward its apex as the successive lateral bundles depart (fig. 3*B*, *D*). Large lateral bundles supplying the carpels are cut off at an acute angle from the periphery of the vascular network in the receptacle slightly above the carpel which they enter. These carpellary bundles pass to the edge of the receptacle, where they abruptly turn in a basipetal direction just before entering the carpellary wall (fig. 3*B*). As the bundle passes through the carpellary wall, it starts to curve gently until it finally attains a horizontal position.

A single carpellary bundle consists of proto- and metaxylem vessels which are distinguished by annular and spiral and reticulate thickenings, respectively (fig. 3*H*). A typical vessel with spiral thickening measures 175 μ by 21 μ. A group of phloem fibers is situated toward the outer side of the vascular strand. These fibers are thick-walled and unlignified. They are of diverse shape but are, in general, elongate with irregular, interlocking ends, and simply pitted. The fibers vary in size from 60 to 210 μ in length by 10 to 15 μ in width. Occasionally secondary vascular tissue is found in the receptacular bundles of mature fruits (fig. 3*I*).

The carpellary bundle enters the carpel as a single strand but divides shortly thereafter into three major bundles. One of these continues in a radial direction as the ovular bundle. The other two diverge to become the dorsal and ventral bundles, respectively. Continued branching of the dorsal carpellary strand gives rise to a series of about six lesser bundles on each side of the carpel (fig. 3*E*). The lesser bundles are parallel to the major bundle and are interconnected by anastomoses, so that a vascular network permeates the carpellary wall. This network lies close to and is parallel with the axis of the seed cavity. Small lateral branches from the network permeate outward into the outer carpellary wall (fig. 3*G*). Vascular elements extend into the peripheral region of the fruit in the distal portion of the carpels and terminate as small strands close to the outer epidermis (fig. 3*F*). Small groups of sclerenchyma cells are found along the inner portion of the pericarp near the receptacle close to the entrance of the vascular supply. These stone cells are somewhat elongated in the direction of the adjacent vascular strands, with thick, lignified walls and simple pits.

ERGASTIC MATERIALS.—Starch is the prominent ergastic material found in nearly all parenchyma of the carpellary wall of the mature, but still firm, fruit. Each parenchyma cell is almost completely filled with starch in the form of simple or compound grains of various sizes (fig. 2*H*). The shape and dimensions of the grains depend upon the number of of grains which compose the cluster. Thus, clusters of large numbers of grains have small grains, while other clusters have one or two large grains (13–18 μ in diameter). Starch is not present in certain parenchyma cells which contain oil droplets, nor in the conspicuous sclereids. Very little starch is observed in the carpellary membrane which surrounds the seed cavity or in the peripheral parenchyma which separates adjacent carpels. There is also relatively less starch in parenchyma of the receptacle than in the carpellary walls.

OIL CELLS.—Scattered throughout the parenchyma of the carpellary wall are conspicuous idioblasts in the form of oil

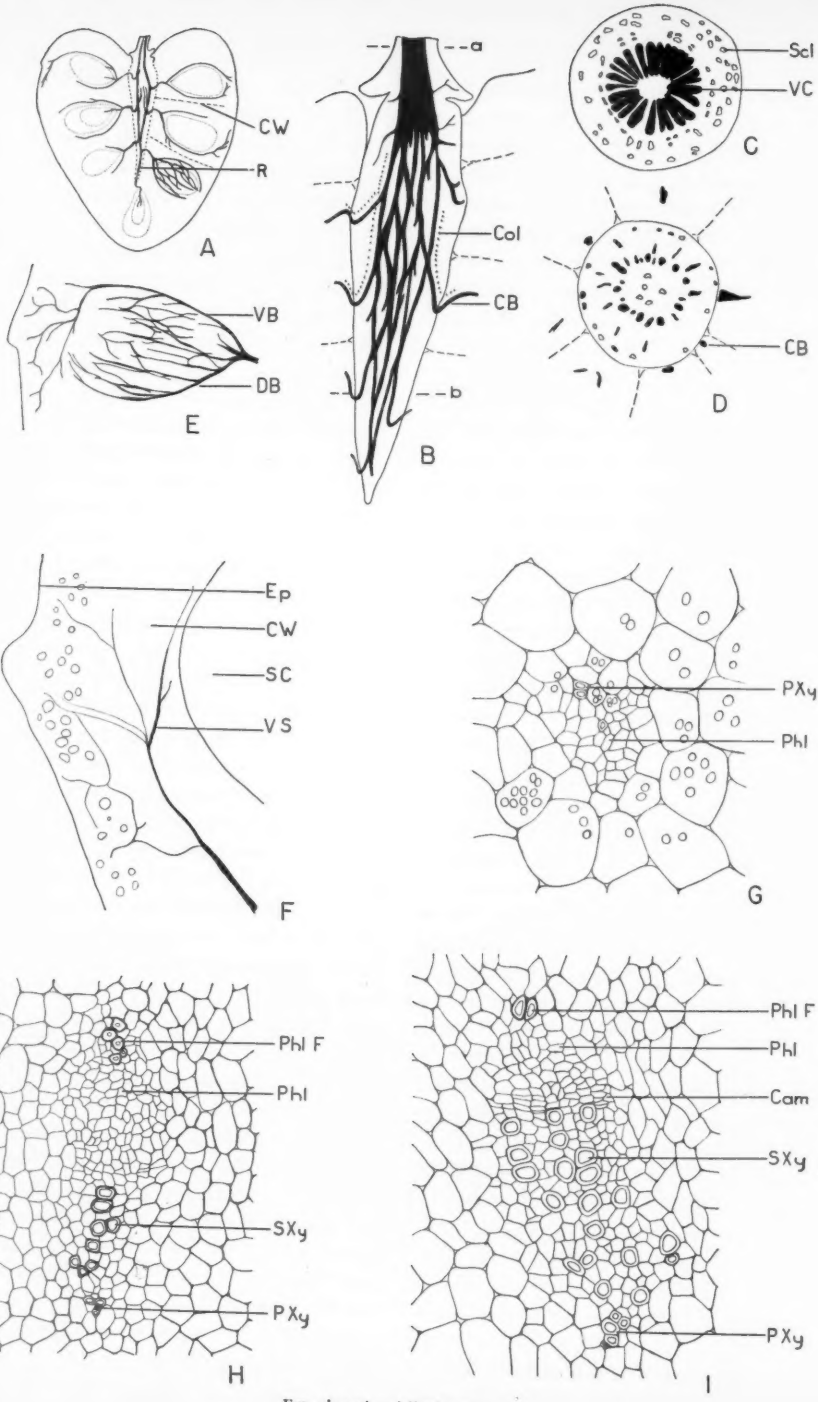

FIG. 3 legend on following page

cells. These are frequently larger than cells of the surrounding parenchyma and measure about 45 μ in diameter (fig. 2*A*). Their cell walls are thicker and more rigid than those of adjacent parenchymatous cells. A single large droplet of oil contained within the cytoplasm stains orange with Sudan III and yellow with iodine. The faintly lignified, thickened cell wall is conspicuous because of its resistance to action of potassium iodide and concentrated sulfuric acid. There is no regularity of distribution of the oil idioblasts. They are found in all parts of the fruit, including the receptacle. In the latter structure the idioblasts, somewhat larger than those in the carpellary walls, attain a diameter of about 80 μ. Similar oil cells are found in the endosperm of the seed.

CHANGES IN STARCH CONTENT.—The most obvious histological change associated with the softening of the fruit is the rapid and complete loss of starch from the carpellary walls. When the fruit is mature but still firm, the carpellary parenchyma which forms the bulk of the fruit is fully packed with starch grains as has been described (fig. 2*I*). When the cool-stored fruit is allowed to soften at 25° C., disappearance of starch is evident even within 9 hours (fig. 2*J*): there is reduction in size of starch grains and number per cell in many cells of the carpellary walls. After 24 hours at 25° C. fewer cells contain irregular numbers of small starch grains. The bulk of the starch by this time has been converted into a soluble substance, presumably sugar. Complete loss of starch, except for occasional small grains, is noted after 48 hours (fig. 2*K*, *L*), at which time the fruit has softened completely and is edible.

SEED

GENERAL MORPHOLOGY.—The cherimoya seed is oval in form and somewhat compressed laterally (fig. 4*A*), the form attained depending in part upon the total number of seeds within the fruit. When many seeds are present, their form is flattened; when very few seeds develop, their form is more rounded. The size of a mature seed is variable—an average being 1.8 cm. long by 1.0 cm. wide and 0.5 cm. thick. The hard, sclerenchymatous, dark-brown seed coat completely surrounds the mass of ruminate white endosperm in which the small embryo is imbedded except at the micropylar end where the remnant of the ovular bundle is found (fig. 4*C*). The only easy access to moisture which the endosperm and embryo have during germination is through the channel provided by the collapse and disintegration of the ovular bundle and the tissue immediately surrounding it. The relatively small embryo is situated very close to this source of moisture (fig. 4*C*).

SEED COAT.—The epidermal layer of the seed consists of thick-walled cells, rectangular in longitudinal section (fig. 4*H*) and isodiametric in surface view. A typical epidermal cell measures approximately 45 μ in length by 14 μ in thick-

FIG. 3.—*A*, longitudinal section through fruit. *B*, longitudinal view of vascular system in receptacle. *C*, *D*, cross sections of vascular cylinder of receptacle at points *a* and *b*. *E*, vascular supply to single carpel. *F*, termination of vascular strands in outer pericarp wall. *G*, cross section of small bundle in pericarp. *H*, cross section of ovular vascular bundle. *I*, cross section of receptacular vascular bundle showing development of cambium. *CW*, carpellary wall; *Ep*, epidermis; *SC*, seed cavity; *VS*, vascular strand; *PXy*, primary xylem; *Phl*, phloem; *SXy*, secondary xylem; *PhlF*, phloem fibers; *Cam*, cambium; *VC*, vascular cylinder; *Col*, collenchyma; *CB*, carpellary bundle; *VB*, ventral bundle; *DB*, dorsal bundle.

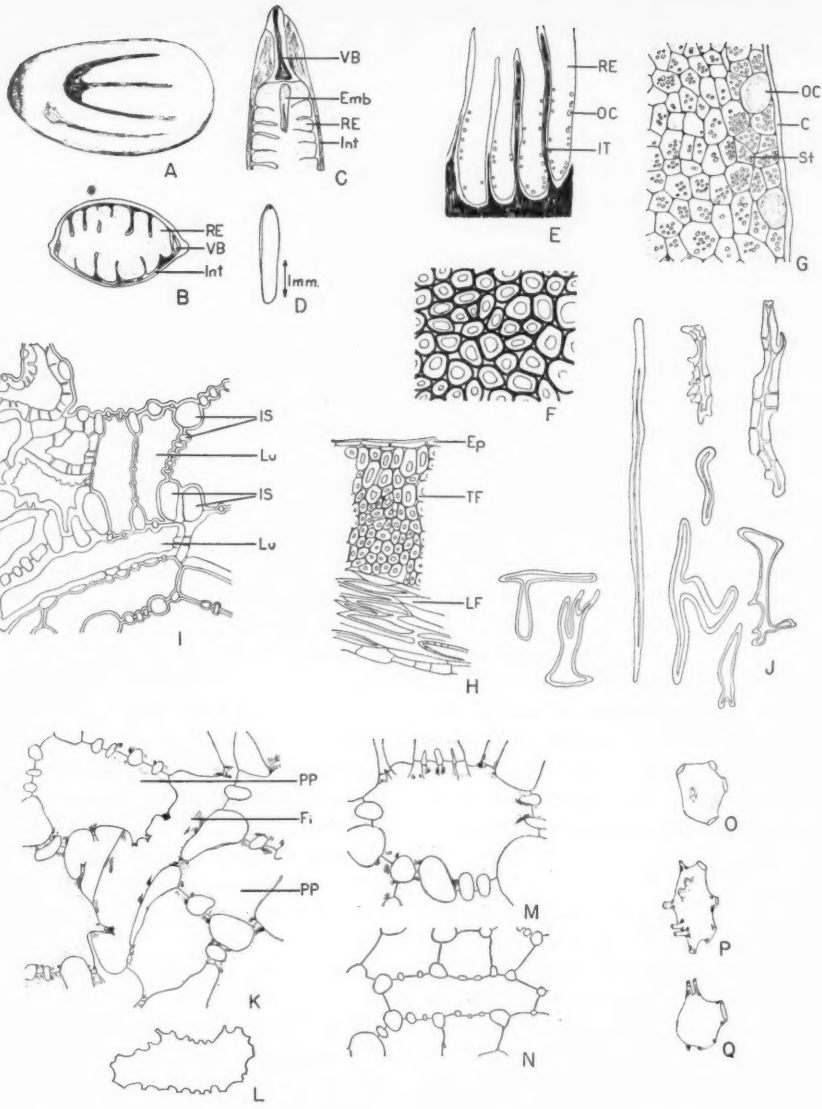

FIG. 4.—*A*, cherimoya seed, surface view. *B*, transverse section. *C*, longitudinal section through micropylar end of seed. *D*, embryo. *E*, diagram of transverse section through portion of endosperm and invagination tissue. *F*, detail of cross section through invagination tissue. *G*, detail of endosperm tissue. *H*, section through seed coat. *I*, tangential view of "packing" parenchyma. *J*, fiber forms from seed coat. *K*, fiber ending. *L–Q*, forms of "packing" parenchyma from cherimoya seed. *RE*, ruminate endosperm; *VB*, vascular bundle; *Int*, integument; *Emb*, embryo; *OC*, oil cell; *IT*, invagination tissue; *C*, cuticle; *St*, starch; *IS*, intercellular space; *Lu*, lumen; *Ep*, epidermis; *TF*, tangential fibers; *LF*, longitudinal fibers; *PP*, "packing" parenchyma; *Fi*, fiber cell.

ness. Immediately beneath the epidermis is a sclerenchymatous layer about ten cells thick, consisting of simple fibers very tightly packed together with their axes parallel to the seed axis. The fiber walls are thick, simply pitted, and heavily lignified. The inner integument lies between this fibrous outer integument and the ruminate endosperm and consists of a layer about 150 μ thick. Its fibers vary in shape from simple elongated cells to those with many branches, and their long axes are at right angles to the seed axis (fig. 4*J*). Fibers of extremely complicated shapes are observed particularly at points near the edge of the radial invaginations where some may be triparted extending along the main axis of the inner integument with radial branches extending into the invaginations. These fiber walls are also thick, simply pitted, and heavily lignified. The lumen is very small in some cases, and the contour of the cell-wall surface very irregular in most instances.

The major portion of the inner integument consists of fiber cells of variable shape which extend inward between the folds of the ruminate endosperm. These fibrous fans or plates are bounded on their surfaces by a layer of unusual "fingered" parenchyma one or two cells deep. The individual cells of the membranes are extremely variable in shape and are characterized by numerous "fingers" of variable length which form the contact points between adjacent cells and fibers (fig. 4*L*). These fingers or extensions are not confined to a given plane but may extend in all directions (fig. 4*K*, *Q*) and are variable in number. Two given adjacent cells may have several points of contact, these points being separated by relatively large intercellular spaces (fig. 4*K*, *M*, *N*). A count of sixty-three cases indicates that a given cell may be in contact in one plane with five to ten other cells. The average frequency of cell-to-cell contacts was six, and the average number of "fingers" for a group of thirty-six cells was 14.9 per cell. One cell was observed to have as many as twenty-four distinct extensions or intercellular connections to adjacent cells (fig. 4*L*) in one plane. The mode of development and the function of this limiting membrane cell layer which covers the fibrous inner integument is not known but is of considerable theoretical interest with respect to surface-to-surface contact points involved in the process of cell division and subsequent cell expansion and differentiation.

Similarly "fingered" cells are found as "packing parenchyma" among the fibers at the points of departure of the radial invaginations into the endosperm.

A heavily lignified layer lines the seed cavity at the hilum end where the ovular bundle enters. This lining extends toward the micropyle end past the point where the embryo is located.

ENDOSPERM.—The endosperm is a white, highly convoluted tissue which comprises the bulk of the seed (fig. 4*B*). It is cellular throughout and is composed primarily of parenchyma, although many idioblasts containing large globules of oil are scattered among the parenchyma, particularly at the periphery (fig. 4*G*). The parenchyma cells have rather thick cellulose walls and are isodiametric cells which measure on the average 64 by 39 μ. The primary ergastic substance found in this tissue is starch occurring in masses of small granules. Fine droplets of a fatty material which is not soluble in xylene or ether, but dissolves in absolute alcohol, are also scattered through the endosperm parenchyma. The spherical idioblast cells are conspicuous because of their relatively large size, approximately

60 μ in diameter, and because they contain a single large globule of oil which stains orange-red with Sudan III and a bright yellow with potassium iodide. Such idioblasts are comparable in all respects to those found in the pericarp parenchyma. The entire outer surface of the endosperm is covered with an epidermal layer of small, thin-walled cells, cuboid in form. Completely enveloping the endosperm is a layer of cutin 3–4 μ in thickness.

Summary

1. Fruits of *Annona cherimola* Mill. can be segregated into horticultural groups based on the nature of the fruit form and surface characters.

2. Considerable variation in density of epidermal hair and stomata is found between fruits of different horticultural varieties and between different parts of the fruit.

3. The bulk of the carpellary wall is parenchyma, the outer portion of which contains sclereid masses. These masses of sclerenchyma have modified parenchymatous cells radiating outward from them.

4. The vascular supply for each carpel is derived from a central reticulate vascular cylinder in the receptacle. Each carpellary bundle divides into three strands upon entering the carpel. One strand supplies the ovule; the other two become the dorsal and ventral bundles.

5. Starch is the prominent ergastic material found throughout the parenchyma in the firm mature fruit but disappears rapidly and completely as the fruit softens. Oil idioblasts are scattered through the parenchyma of the carpellary wall and of the endosperm of the seed.

6. The seed coat consists of heavily lignified, sclerenchymatous layers. The inner surface of the inner integument is characterized by a layer, one or two cells in thickness, in which the cells show extreme variability in shape and are separated by conspicuously large intercellular spaces.

7. The cells of the convolute endosperm are filled primarily with starch, although a number of oil idioblasts occur, particularly near the periphery.

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STUDIES OF FLORAL MORPHOLOGY IN THE ERICALES. I. ORGANOGRAPHY AND VASCULAR ANATOMY IN THE ANDROMEDEAE[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 626

BARBARA F. PALSER

Introduction

Among the angiosperms certain plants display such consistency in the possession of a particular array of characters that they have long been recognized as a natural group. Such an assemblage is the nucleus of the Ericales. To be sure, certain small families such as the Diapensiaceae, Lennoaceae, and Empetraceae have been added or subtracted from the order at various times by different taxonomists, but the central group consisting of the Clethraceae, Ericaceae (including the Pyrolaceae, Monotropaceae, and Vacciniaceae, which have been considered by some to have family rank), and the Epacridaceae has been maintained as a separate order over a very long period of time.

In spite of the naturalness of the assemblage there occurs within it the change from certain characters that are considered primitive—actinomorphy, hypogyny, pentacycly, polypetaly, freedom of stamens from corolla, and hermaphroditism—to the contrasting characters which are considered advanced—zygomorphy, epigyny, tetracycly, sympetaly, the epipetalous condition of stamens, at least functional dioecism, and a decrease in total number of parts per whorl of the various floral organs. This suggested that a detailed study of the group might shed some light on the possible origin of these advanced characters.

Although recognition of a relationship among the various members of the Ericales has been relatively easy, determination of their relations with other groups has not been so clear. They have variously been assigned positions suggesting derivation from the Theales (**8, 12, 35**), from the general area of the Dilleniales (**36**), from the Caryophyllales close to the Primulales (**5**), and as the lowest order of the Sympetaleae arising just above the level of the Rosales and giving rise to the Primulales and Ebenales; and the Ericaceae have been placed at the end of a side branch which also included the Empetraceae and Polygalaceae, this branch arising from a main branch going to the Anacardiaceae, Sapindaceae, and Aceraceae just above the Simarubaceae and Burseraceae (**48**). Not only have the interordinal relationships proved difficult but the actual classification within the order has met many snags: Should the small families mentioned earlier be included in the order? How many families should be recognized? Into what subfamilies, tribes, and genera should these families be divided? Into what genera should many species be placed? And how has the group attained its present worldwide distribution? When, using the usual morphological characters, taxonomists have not succeeded in arriving at satisfactory answers to many of these questions, study of less obvious structures—such as vegetative anatomy, particularly of the wood, chromosome numbers, detailed floral characteristics, vascular

[1] This work was supported in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

anatomy, and embryology in the broad sense—should be undertaken to see if they can offer any evidence in support of one system of classification or another.

Some studies of one or another of these areas have been made in the Ericales, certain authors making no attempt to apply the results to the problems suggested above, others doing so. In his classic work on organogeny PAYER (**42**) described the floral development of a few species. PELTRISOT (**43**) considered certain aspects of seed development of several species, and ARTOPOEUS (**1**) made some observations on this same subject but paid more attention to a study of the structure and dehiscence mechanism of the stamens. In this latter he was followed by MATTHEWS and KNOX (**38**). SAMUELSSON (**44**) studied several developmental aspects of the seeds of a large number of species and on this basis decided that the Empetraceae definitely belong in the Ericales, while the Diapensiaceae are not so closely related to most of the order as had been thought by many. This opinion as to the position of the Empetraceae has been corroborated more recently by HAGERUP (**31**) on the basis of a fairly thorough anatomical study. A thesis by HANCY (**33**) described the vascular anatomy of a few hypogynous and epigynous species of the Ericaceae. BREITFELD (**6**) on the Rhododendroideae and NIEDENZU (**40**) on the Arbutoideae and Vaccinioideae have attempted to apply a study of leaf anatomy to the taxonomy of these respective groups. HAGERUP (**30**) has determined the chromosome numbers of many of the species to see if they would help in determining relationships. More recently CAMP (**9–11**), COPELAND (**13–22**), and COX (**23, 24**) have been making a determined attack on various aspects and various sections of the order. CAMP has, for example, applied chromosome numbers, population aspects, and geographic distribution to unraveling the speciation in *Vaccinium*. COX has used his findings in wood anatomy in formulating a system of classification for two subfamilies of the Ericaceae, the Rhododendroideae and the Arbutoideae. COPELAND has studied several aspects of floral morphology in certain groups. On the basis of studies of *Pholisma* he is of the opinion that the Lennoaceae should not be included in the Ericales. His studies on the Pyroleae, Monotropoideae, and Rhododendroideae, which include some vegetative anatomy and work on the flower—vascular anatomy, stamen structure, and ovule and seed development—have led him to propose classification systems for these groups. He is at present working on the Arbuteae.

Even with all these papers, and others less comprehensive, a great deal remains to be done to arrive at a knowledge of the order complete enough to answer at all satisfactorily some of the problems suggested above. The author hopes to join those already working on the group and has chosen the Andromedeae as the initial point of attack.

There are several reasons for choosing the Andromedeae. This tribe has by some been considered among the more primitive of the Ericaceae proper, and it has been suggested that this group may have been ancestral to some of the derived forms (**22**). Certain genera of the tribe have been distinct for a long time—*Oxydendrum*, *Epigaea* (sometimes placed in a separate tribe), and to a certain extent *Chamaedaphne* of the native genera, and probably also *Enkianthus*, *Agauria*, and *Orphanidesia*, which are not native in America. The remaining species, forming two groups, have been included within each group in one to several genera

and, where several genera are distinguished, have been shifted around among certain of these genera, so that author names frequently are a necessity in citing the scientific name of a species. In one group are found those species either placed in *Cassiope* or subdivided into *Cassiope*, *Harrimanella*, and *Arcterica*. The other is a larger group and includes such genera as *Andromeda*, *Ampelothamnus*, *Lyonia*, *Leucothoe*, *Eubotrys*, *Arsenococcus*, *Desmothamnus*, *Pieris*, *Neopieris*, *Xolisma*, *Zenobia*, and *Oreocallis*. Of these, *Andromeda*—although almost every species in the whole tribe has been placed in this genus at some time—has emerged relatively distinct, but the synonomy among the others is difficult to unravel.

In general, members of the Andromedeae are so geographically distributed that it is easier for a Chicagoan to obtain this material than for many of the other groups; several are found in the North Temperate to circumpolar regions, and others are located in mountains or coastal plains of the southeastern United States. Only three genera, *Enkianthus*, *Orphanidesia*, and *Agauria*, are not natively found in this country, the former two being Asiatic and the latter African.

Stamens in the Andromedeae show considerable variation in appendages—a feature characteristic of so many of the Ericaceae as to have earned the name Bicornes as an ordinal epithet. Certain stamens lack appendages, but others have them, ranging greatly in size and of two general types: downward and outwardly extending spurs and awns which are continuations of sterile tissue above the thecae of the anthers. The exact nature of these appendages is not understood and will require detailed developmental studies to elucidate.

Because of the vast amount of material that would be contained if a complete discussion of the members of the tribe were undertaken and because complete developmental material is difficult to obtain, this first paper deals only with certain facts of the general organography and vascular anatomy of flowers of those species for which suitable material could be obtained.

Material and methods

Of the various members of the tribe Andromedeae, only three species are found within easy access of Chicago, and only one of these in any abundance. Consequently, collections of material were made on scattered trips taken for the purpose and by friends and colleagues who were kind enough to help. The names used in the descriptions throughout will be those used by the collector or the name used for the first collection of any species. For material collected by the writer the names are those found in the new *Gray's Manual* (**28**) as far as possible. This system does not always appear completely consistent but is the most expedient. For certain plants bought from nurseries and grown in the greenhouse, the name is that used by the nursery. Table 1 is a summary of the species studied and the source of the material. Certain of the collections, though not made by Dr. HERBERT F. COPELAND himself, were sent to me by him.

The genera *Gaultheria*, *Pernettya*, and *Chiogenes*, which are sometimes included in the Andromedeae and sometimes placed in a separate tribe, the Gaultherieae, will not be considered in the present study. Three species of the first genus are being studied by Miss CHOU, and her results will be published at some future date.

Most collections were killed and fixed

TABLE 1

Species	General area where collection was made	Collector
Ampelothamnus phyllireifolius (Hook.) Small	Georgia	CAMP[a] and DUNCAN
Andromeda glaucophylla Link	Ithaca, N.Y.; northern and southern Michigan; northern New Hampshire; greenhouse	CAMP; BRITTON;[b] nursery—KELSEY;[c] GALL[d] and PALSER; HARRIS[e] and PALSER
Andromeda polifolia L.	Near Juneau, Alaska	JUNEAU BOTANICAL CLUB[f]
Cassiope hypnoides D. Don	Mt. Washington, N.H.	HARRIS AND PALSER
Cassiope lycopodioides (Pall.) D. Don	Near Juneau, Alaska	JUNEAU BOTANICAL CLUB
Cassiope mertensiana (Bong.) G. Don	Mt. Rainier Nat'l Park,* Wash.; near Juneau, Alaska	JUNEAU BOTANICAL CLUB; PALSER
Cassiope tetragona (L.) D. Don	Eagle Summit, Alaska	JUNEAU BOTANICAL CLUB
Chamaedaphne calyculata (L.) Moench	Ohio; Ithaca, N.Y.; northern and southern Michigan; northern New Hampshire	CAMP; BRITTON; GALL; GALL and PALSER; HARRIS and PALSER
Enkianthus campanulatus (Miq.) Nichols	Greenhouse	Nursery—KELSEY
Enkianthus cernuus (Sieb. & Zucc.) Makino	Strybing Arboretum, Golden Gate Park,* Calif.	KAVALJIAN[g]
Epigaea repens L.	Northern Michigan; Great Smoky Mts. Nat'l Park,* Tenn.; Ohio; Ithaca, N.Y.; northern New Hampshire; northern Indiana	CAMP; SHANKS;[h] PALSER; GALL and PALSER; HARRIS and PALSER
Harrimanella stelleriana (Pall.) Coville	Mt. Ararat, Mt. Rainier Nat'l Park,* Wash.; near Juneau and Mt. Roberts, Alaska	CAMP; JUNEAU BOTANICAL CLUB
Leucothoe axillaris (Lam.) D. Don	Near Tallahassee, Fla.	LAMP[i]
Leucothoe catesbaei (Walt.) A. Gray	Great Smoky Mts. Nat'l Park,* Tenn.; greenhouse	TAYLOR;[j] PALSER; CAMP; nursery—ROBBINS[k]
Leucothoe racemosa (L.) A. Gray	St. Mark's Wildlife Reserve, Fla.	LAMP
Leucothoe recurva (Buckl.) A. Gray	Greenhouse	Nursery—KELSEY
Lyonia ligustrina (L.) DC.	Great Smoky Mt. Nat'l Park,* Tenn.; greenhouse; Ithaca, N.Y.	TAYLOR; PALSER; CAMP; nursery—KELSEY
Lyonia mariana (L.) D. Don	Greenhouse	Nursery—KELSEY
Neopieris nitida (Bartr.) Britton	Georgia; St. Mark's Wildlife Reserve, Fla.	CAMP and DUNCAN; LAMP
Oxydendrum arboreum (L.) DC.	Ohio; Great Smoky Mts. Nat'l Park,* Tenn.	CAMP; SHARP;[l] PALSER
Pieris floribunda (Pursh) Benth. & Hook.	Corvallis, Ore.—cultivated; Great Smoky Mts. Nat'l Park,* Tenn.; greenhouse	KRAUS;[m] PALSER; nurseries—KELSEY, ROBBINS
Pieris japonica (Thunb.) D. Don	Sacramento, Calif.—cultivated; Corvallis, Ore.—cultivated; greenhouse	COPELAND;[n] KRAUS; nurseries—KELSEY, VAN DER POL[o]
Xolisma fruticosa (Michx.) Nash.	St. Mark's Wildlife Reserve, Fla.	LAMP

* Collections in the National Parks were made with the permission of the Park Naturalists: Mr. RUSSELL GRATER at Mount Rainier and Mr. ARTHUR STUPKA at Great Smoky Mountains; at Golden Gate Park they were made with the permission of the supervisor of the Arboretum, Mr. ERIC WALTHER.

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in Conant's modification of Navashin's solution, but a few other solutions were used: formalin–acetic alcohol, Bouin's, and other modifications of Navashin's. All were dehydrated in an ethyl alcohol–tertiary butyl alcohol series and embedded in a paraffin-rubber-beeswax mixture. For most buds and flowers a paraffin melting at 55° C. was used, but for some winter buds with heavy scales, some flower buds with hard calyxes, and some fruits a paraffin melting at 60°–62° C., or similar tissumat, was employed. Cross and longitudinal series were cut, usually at 10 μ, though a few were at 8 μ, 12 μ, or 15 μ. As few as four flowers in one species and as many as fifty-six in another were cut for study, but the average number was about fifteen. Three different stains were used: safranin and light green, KRAUS' modification of Flemming's triple, and FOSTER's (29) tannic acid–ferric chloride and safranin. The latter proved the most satisfactory for all-around use. Care was needed in staining and destaining to prevent obscuring of interesting features by the large amounts of highly stainable materials presumed to be tanniniferous. All drawings were made with a camera lucida, but some of the longitudinal diagrams have had some details added from adjacent sections in the series.

Observations

There is enough similarity in the various species studied to indicate relationship and to make possible a generalized description. Thus, the descriptions for each species point out only its peculiarities. In a few instances a complete description is not possible because the material available was only very young buds or fruits. Descriptions of organography are based on sections rather than on fresh material, as collections were either received already in killing solution or were made under conditions not conducive to detailed study. They are intended to supplement, not to replace, standard morphological descriptions. Descriptions of vascular anatomy treat of the pathways of the vascular tissues, not of the details of cellular structure of individual bundles. In both cases most of the descriptions are taken from buds just before opening, supplemented for most species by both younger and older material.

ORGANOGRAPHY

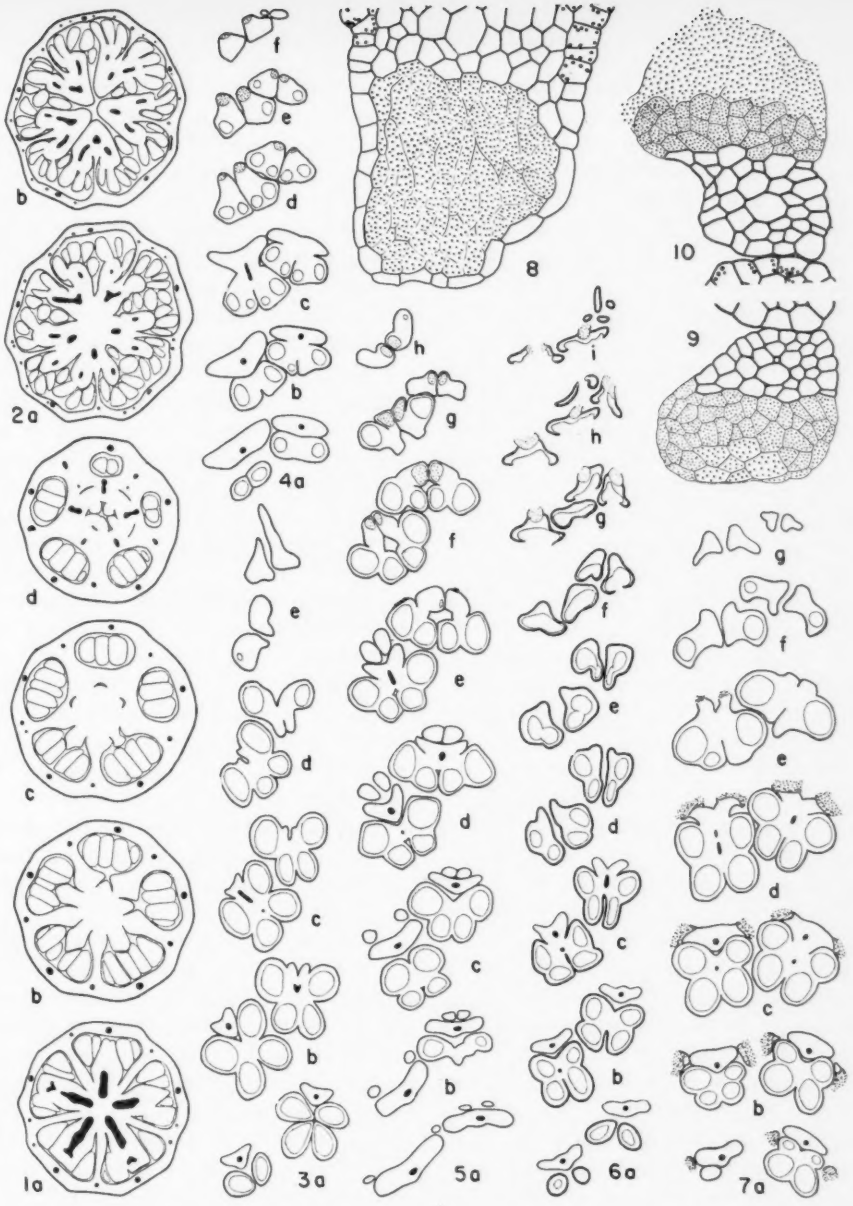
GENERAL.—The pedicel of the flower may (figs. 11, 19–21) or may not be bracteate. The flower is pentacyclic, pentamerous, actinomorphic, sympetalous, hypogynous, and perfect except for *Epigaea repens*, which is functionally dioecious. The five sepals are in most cases completely diverged from one another from their origin but in some species may be undiverged for a short distance at the base. In many species the sepals are turned downward for a greater or lesser distance from their point of origin before ascending in a more normal position (figs. 13, 15, 54–56). The sepals of a few are more or less covered with hairs. Aestivation in the bud is varied: imbricate (here restricted to that imbricate arrangement in which one sepal is wholly internal, one wholly external, and three are half internal and half external), quincuncial (figs. 25, 53) (that type of imbricate aestivation in which two sepals are wholly internal, two wholly external, and one half internal and half external), valvate (figs. 42, 59), and reduplicate.

The five petals alternate with the sepals and, in all species, are undiverged from one another from their point of origin for from about one-half to almost their entire length. The undiverged co-

rolla shows valvate, induplicate (fig. 59), or reduplicate aestivation, while the free limbs may be valvate, imbricate, or quincuncial. The shape of the corolla is varied from a somewhat funnel-shaped to a bell-shaped, to a somewhat urceolate (fig. 17), to a very pronounced urceolate (fig. 16) condition. These terms are used in the following senses: funnel-shaped—the mouth broader than the tube; bell-shaped—the mouth and tube approximately the same diameter; and urceolate—the mouth smaller than the tube in diameter. The opening of the corolla correspondingly varies from fairly broad to extremely narrow. The base of the corolla may be somewhat reflexed and in a few species may have decided sacs bulging between the sepals at the base. Rarely the corolla bears hairs on one surface or the other.

The ten stamens occur in two whorls of five each. The outer whorl, lower in point of origin, is opposite the petals, while the inner, or upper, is opposite the sepals. The filaments vary in cross section from almost round to considerably flattened laterally. They may show short regions of considerable enlargement near the base (figs. 16, 18), usually the result of radial enlargement of a few cells, or, in one case at least, may be undiverged from the petals for a short distance. They meet the introrse anthers somewhere near a midpoint in most species (figs. 3–7, 12, 15, 18), but in one group it is near or at the top (fig. 13—in this figure the anthers have not yet inverted). It has been quite clearly shown by ARTOPOEUS (1) and MATTHEWS and KNOX (38) that the introrse condition is acquired during development, i.e., that the apparent apex of the anther is morphologically the base and the apparent base, the morphological apex. Sections of very young flowers of several of the present species have corroborated this finding. For convenience, however, the terms "apex" and "base," etc., will be used in the apparent or positional sense, not in the true morphological sense. The filaments may be relatively straight or may form an S-curve between the base and anther, the S either vertical (S), or horizontal (ᔕ) (figs. 16, 18). The anthers contain four thecae, two on each side of the connective (figs. 3–7). In older anthers the two on each side become confluent, so that each shedding anther has only two pollen sacs. The total length of the anther, the relative lengths of the two thecae in an anther half, and the degree of separation into the two halves are varied. The microspores in all species do not separate following meiosis, so that the shedding pollen is in the form of tetrahedral tetrads. The position of the anthers in the flower is varied, from just above the top of the ovary (fig. 15) to almost at the corolla opening. No anthers were found which project beyond the opening.

That stamen character which is peculiar to the Ericaceae, possession of appendages, is well illustrated in the Andromedeae. These appendages have been variously named, but two terms will be used here to denote the two general types of appendages which are found: "spur" and "awn." "Spur" will be used for those appendages, two per stamen, of somewhat varying length and character which arise from the filament, the connective, or the anther itself close to the region of filament-anther junction (figs. 5, 7). "Awn" will be used for the continuation of sterile tissue beyond the top of the thecae of the anther (figs. 3, 6, 15). Stamens may show neither, one or the other, or both of these types of appendages. Some spurs are composed in part of a peculiar type of tissue, including the epi-



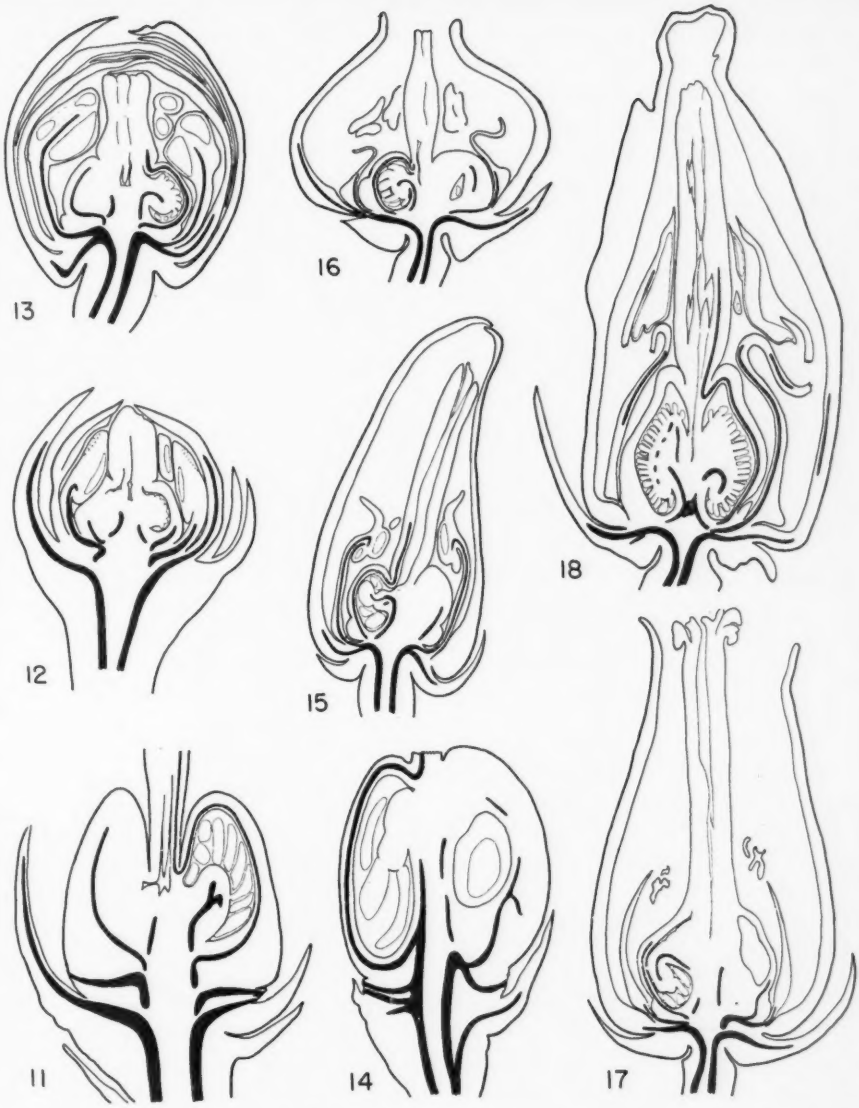
FIGS. 1–10.—Fig. 1*a–d*, *Chamaedaphne calyculata*, bud, ×33. Serial cross sections of ovary from region of placental attachment to base of depression. Fig. 2*a*, *b*, *Epigaea repens*, bud, ×20. Cross sections of ovary, near base and near top. Figs. 3–7, serial cross sections of stamens, sepaladl stamen to left, petalad to right. Fig. 3, *Andromeda glaucophylla*, bud, ×23. Fig. 4, *Oxydendrum arboreum*, very young bud, ×31. Fig. 5, *Ampelothamnus phyllireifolius*, bud, ×20. Fig. 6, *Leucothoe recurva*, old bud, ×33. Fig. 7, *Lyonia mariana*, young bud, ×31. Single hatched area—highly cytoplasmic cells; stippled area—granular material. Fig. 8, *Ampelothamnus phyllireifolius*, ×332. Enlargement of granular pouch area in connective of sepalad stamen as in fig. 5*f*. Figs. 9, 10, *Neopieris nitida*, bud, ×332. Detail of spurs close to filament at two different ages; comparable to fig. 7*b*. Stippled cells are highly cytoplasmic; stippled region—a nonstaining granular material.

dermal cells, which degenerates early in development (figs. 7*a*, *b*, 9, 10). This tissue usually continues above the juncture of the spurs with the filament, so that the filaments have a disintegrating margin (fig. 7*c*). Above the juncture of these filaments with the anthers projecting areas of the connective also show the same disintegrating regions (figs. 7*c–e*). In certain other species where the spurs show no disintegrating areas, masses of connective tissue continue above the point of junction of anther and filament. Toward the anther top each protruding area of connective develops a pouch of cells, covered by the epidermis, which become highly granular and then disintegrate (figs. 5, 8). The epidermis cracks, and the granular mass appears to be discharged, so that on older anthers only the margins of the empty sacs remain. Some species having no spurs show a region of disintegrating tissue similar to that found on the spurs but in a position at the edge of the connective near the anther top (figs. 4, 6). Whether the disintegrating tissues of spurs and upper connective tissue and the granular pouches are essentially the same will require a more thorough developmental study. Short awns may be crushed in part during later stages of development, so they are not so recognizable in mature anthers as in young ones. Occasionally, however, the awns are quite long and may be two or four per anther (figs. 3, 6, 15). No attempt was made to determine details of anther dehiscence, but it is typically by apical pores or short slits.

The stamens develop early, and, in the many species in which the flowers are initiated in summer or fall preceding the year in which they open, the anthers normally pass winter in the microspore mother-cell stage and reach the tetrad stage before ovules have developed past the megaspore mother-cell stage.

The ovary is in most cases essentially globular, usually depressed at the top (figs. 11, 14–16, 18), with the style springing from the base of the depression. The style is straight (figs. 15, 17) or may bulge somewhat above the ovary (figs. 16, 18) and terminates in the stigma at about the level of the corolla opening (except in "staminate" flowers of *Epigaea*). The stigma may be narrower than the style (fig. 16), about the same width (fig. 15), or considerably enlarged (fig. 17); it may show no obvious lobing or may be conspicuously lobed. The ovary is five-loculed, the locules being opposite the petals (figs. 30, 43, 53, 62). There is a central column of tissue, at least in the ovary base, and in some cases essentially to the top; the placentae, five (figs. 1, 31) or ten (fig. 2*a*), arise from this, so that the placentation is axile, at least at the base. In many species the central column shows a split at the top (figs. 31, 32); this split is continuous through the middle of the top of the placentae—if they were not already in two parts—making the placentation in the upper portions parietal (fig. 2*b*), or essentially so, although placentae are extended very deeply into the locule cavity.

Placentae vary in size from those which occupy one-quarter or less of the carpel cavity (fig. 15) to those which appear to occupy almost the entire area (fig. 18). In most species ovules are borne on the outer surface of the placenta between it and the dorsal wall (figs. 11–16, 18), but in a few the ovules occur on the inner surface only, between the placenta and the central column (figs. 17, 30, 53). The number of ovules, and correspondingly their size, varies. In some species as few as ten or twelve ovules may be found in a locule (fig. 14), while in one the number may approach two hundred (fig. 18). All ovules in the group would be considered relatively small. The



FIGS. 11–18.—Diagrams of longitudinal sections. Fig. 11, *Oxydendrum arboreum*, young fruit, ×19. Fig. 12, *O. arboreum*, very young bud, ×25. Note elongation of receptacle between this age and the previous. Fig. 13, *Cassiope mertensiana*, young bud, ×24. Note that at this age anthers are not inverted or even horizontal; reflexion is not so great as in older buds and flowers. Fig. 14, *Enkianthus cernuus*, fruit, ×11. Fig. 15, *Andromeda glaucophylla*, bud, ×11. Fig. 16, *Lyonia ligustrina*, flower, ×11. Fig. 17, *Leucothoe axillaris*, flower, ×11. Fig. 18, *Lyonia mariana*, old bud, ×11.

ovules are unitegmic, tenuinucellate, and closer to anatropous than anything else. They are not strictly anatropous, however, as there is a definite bend at the chalazal end of the megagametophyte in most species.

The central split which connects the five locules of the ovary is continuous into and through the style, forming a five-angled stylar canal (fig. 15), the angles of the canal corresponding to the locules and continuous with them, and the tissues between angles of the canal corresponding to the septa between the carpels. In young buds the stylar canal is almost completely closed but opens rather widely in older flowers (fig. 17). In a few species the cells lining the canal become loose, so that the latter appears to contain a sort of aerenchyma tissue. The surface of the stigma is usually characterized by loose, papillate cells.

The ovary surface may, in some species, be covered with hairs to a greater or lesser extent. These are normally denser in young flowers than in older ones.

The group as a whole appears to be characterized by a certain amount of instability in numbers of floral parts. This instability is with respect to the number of members per whorl rather than to number of whorls. The only approach to an exception to this statement so far observed is in the "pistillate" flower of *Epigaea repens*. Here the stamens are replaced by staminodes, but normally ten of these are found, and, while they arise very close together, they can probably best be interpreted as arising in two whorls. The number of members per whorl can be varied in any one, or more than one, of the whorls, and examples have been observed of variation both up and down, i.e., six or four.

Most members of the group studied characteristically possess nectariferous tissue—little in some species, much in others. The nature of the nectary varies, though it is generally associated with the base of the ovary. The available material of one or two species showed no nectariferous cells (fig. 14), but this needs to be checked by additional material of different ages. The simplest nectaries consist of a few glandular cells in an otherwise unchanged ovary wall; others show no free nectary lobes but a definite nectariferous enlargement of the ovary base (figs. 16, 52). In some these may become ten-lobed, and small free areas of nectariferous tissue project upward. In some the region of attachment to the ovary is reduced, and a fairly extensive ring of tissue or free lobes extend upward between the filaments of the stamens (figs. 18, 30). In some species with a definite downward reflexion at the base of the flower, downward lobes of nectariferous tissue occur (figs. 56–59) as well as the upward ones; still other species have, between the stamens, nectaries which have little or no connection with the ovary but appear to arise from the receptacle.

INDIVIDUAL SPECIES.—*Ampelothamnus phyllireifolius* (Hook.) Small. (*Andromeda phyllireifolia* Hook., *Pieris phyllireifolia* [Hook] DC.).—The massive sepals show valvate aestivation. The corolla is long, somewhat urceolate, with short free limbs. The filaments show enlargements at the base and are vertically S-shaped below the region of anther attachment. Large downwardly and outwardly extending spurs arise from the connectives, which higher are characterized by granular pouches (figs. 5, 8). Anthers are located close against the style just below the stigma. The ovary is somewhat irregularly globular, deeply depressed, and the style is essentially straight and bears an unenlarged stigma.

Each medium-sized placenta bears thirty to forty ovules on its outer surface. Nectariferous tissue occurs at the ovary base and extends upward as ten free lobes between the stamen filaments.

Andromeda glaucophylla Link (this is considered by many as only a race or variety of *A. polifolia* L.) (figs. 3, 15, 54–62).—The sepals and the corolla show a fairly strong reflexion at the base. The rather small sepals remain undiverged for a short distance and above show valvate aestivation. The corolla is strongly urceolate with only short recurved free limbs. In the bud the base of the corolla shows a pronounced induplicate aestivation. The filaments have a slight basal enlargement and are essentially straight and short; the anthers are located just above the ovary. Two fairly long awns per anther project upward and outward. The style is fairly massive and terminates in a slightly expanded stigma. The placentae are small and axile throughout as the connection of the locules to the stylar canal occurs above the placentae. The ovules, twelve to eighteen per locule, are fairly large and attached to the outer and lower surfaces of the placenta. The nectariferous tissue is at the ovary base, with ten free downward lobes and continuing upward as ten and then five enlarged areas of the ovary wall.

Andromeda polifolia L.—The organography of the flower is essentially the same as that of *A. glaucophylla*. A minor difference is that there is little or no free downward lobing of the nectariferous tissue.

Cassiope hypnoides (L.) D. Don. (*Andromeda hypnoides* L., *Harrimanella hypnoides* [L.] Coville).—The only material available of this plant was a collection made just subsequent to pollination and fertilization, so that petals and stamens had begun to wither and fall off and were not present on many flowers. The floral parts, particularly the sepals and corolla, are strongly reflexed at the base. Sepals are small and undiverged from one another for a distance, and the free limbs show an essentially valvate aestivation, though this might be overlapped in the bud. The corolla appears to be somewhat bell-shaped, the base showing induplicate aestivation, while tips of the petals, which are free for a considerable distance, are imbricate. Stamens are characterized by long, thin, downwardly and outwardly projecting spurs. These arise either from lateral edges of the filament just below the region of filament-anther junction or from the anther wall at the same level. This juncture is near the anther top rather than at a midpoint, and the anther is not completely vertical. The style broadens above the depression and tapers to an unenlarged stigma. The seventy to eighty rather small ovules per locule are borne on the outer and basal surfaces of the placenta, which has a large downward lobe. The nectariferous tissue occurs at the base of the ovary where it forms ten enlarged areas which have a short free upward projection.

Cassiope lycopodioides (Pall.) D. Don. (*Andromeda lycopodioides* Pall.)—This species is similar in many respects to *C. hypnoides*, but certain differences do occur. The sepals are completely diverged and show quincuncial aestivation. The bell-shaped corolla has only short free limbs which are quincuncial, and the base is not induplicate. The anthers are not vertical but tipped downward at an angle from the filament toward the style. The spurs show regions of disintegration which continue above in the connective. The style is essentially straight. The nectary, attached to the base of the ovary, becomes a continuous free ring above.

This ring gives rise to five lobes at a still higher level.

Cassiope mertensiana (Bong.) G. Don. (*Andromeda mertensiana* Bong., *Andromeda cupressina* Hook., *Cassiope mertensiana* vars. *gracilis*, *ciliota*, *californica* Piper) (fig. 13).—*C. mertensiana* is similar to *C. lycopodioides* with a few minor differences. Floral parts are more strongly reflexed, and sepals are undiverged for a short distance, their free limbs valvate. There appears to be no enlargement of the filaments which join the anthers at their upper extremity. The anther position is horizontal or may occasionally even be slightly tipped upward in the old bud or flower which would make these slightly extrorse rather than introrse. In the young bud they are only slightly tipped inward. The spurs arise laterally from the filament and show regions of disintegration which in this species appear to be associated with dehiscence of the anther. The stigma is slightly enlarged and somewhat vaguely ten-lobed. The continuous nectary ring arising from the ovary is ten-lobed above, the lobes between the stamens.

Cassiope tetragona (L.) D. Don. (*Andromeda tetragona* L.).—This is similar to *C. mertensiana*. The aestivation of the free limb of the sepals may be quincuncial in the bud. The anther position is slightly tilted down from the horizontal. The stigma is not enlarged. The free lobes of nectariferous tissue above the ring are first five and then ten, the latter occurring in pairs close on either side of the filaments of the sepalad stamens. Nectariferous tissue also appears to extend somewhat lower in the flower, being found on receptacle as well as ovary base.

Chamaedaphne calyculata (L.) *Moench.* (*Andromeda calyculata* L., *Lyonia calyculata* [L.] Reichenb., *Cassandra calyculata* [L.] D. Don).—Two large bracts occur immediately below the flower and persist. The massive sepals show quincuncial aestivation. The slightly urceolate corolla has short free limbs which are imbricated in the bud. Filaments are considerably enlarged above the base, and each anther, located just below the corolla opening, is topped by two short awns. The style, with an enlargement just above the ovary, extends beyond the corolla opening to terminate in a slightly expanded stigma. The relatively large placentae are axile, and there appears to be only a small rather indirect connection between locules of the carpels and the stylar canal (fig. 1*a–d*). The few large ovules, approximately ten per locule, are borne on the sides and toward the base and top of the placentae. The nectary lobes, ten large and long ones, extend from the ovary base up between the stamen filaments. Four-carpellate flowers have been seen and also ovaries basically five-carpellate but with a septum missing. Perhaps even more interesting is the suggestion of development toward unisexuality. Some flowers appeared perfect, both stamens and ovules apparently functional. In others, however, one to all locules showed all ovules aborted, while the stamens were functional; in still other flowers, where the ovules were functional, many or all of the anthers showed aborted contents.

Enkianthus campanulatus (Miq.) Nichols. (*Andromeda campanulata* Miq.).—One specimen planted in the greenhouse started fairly well. Buds collected proved, when sectioned, to be only vegetative. In the second spring, although some buds started, they soon dried and fell off. A few buds collected at this time proved, when sectioned, to be floral but were so badly shriveled that structure was not discernible.

Enkianthus cernuus (Sieb. & Zucc.) Makino. (*Meisteria cernua* Sieb. & Zucc.) (fig. 14).—Only one collection, made during September, was obtained; this contained fruits and winter buds. The latter proved almost impossible to cut, and the sections obtained showed no signs of floral buds. Certain facts of organography can be inferred from a fruit, but many details are not known, particularly of corolla, stamens, style, and stigma. The sepals, fairly heavy and valvate in the fruit, are not reflexed. The relatively small placentae arise near the top of the locules, and each has a long, downward lobe. The few ovules, approximately ten to fifteen per locule, are borne on the outer and basal surfaces of the placenta. There was a slight indication of a region of nectariferous tissue at the ovary base, but this needs confirmation from much younger material.

Epigaea repens L.—This species was for a long time thought to be heterostylous, but STEVENS (**46**) has conclusively shown it to be functionally dioecious. By some peculiar coincidence, most of the material in the various collections available appears to be "pistillate." Out of fifty-six buds, flowers, and young fruits sectioned, only three showed any signs of functional stamens; of these, one was past flowering and so shriveled and dried as not to be useful; the other two were very young buds collected in September. One of these appeared abnormal and cut poorly, so that critical sections were lost. The description of the "staminate" flower is therefore based on a single young bud. The two types of flowers are similar in many respects, the differences occurring in the stamens and in the style and stigma of the pistil. The flowers are closely invested by three large bracts arising from the pedicel just below the flower, one opposite the inflorescence stalk, the other two essentially opposite each other at right angles to the plane of the first. The sepals are quite broad and long and show quincuncial aestivation. The corolla is funnel-shaped with quite long free limbs which are also quincuncial. In buds the inner surface of the corolla, particularly of the "pistillate" flowers, is closely beset with peculiar hairs. In the "pistillate" flower the stamens are replaced by staminodes, similar in position and shape to the filaments but smaller and usually terminating below the level of the ovary top. Length of the stamens in the "staminate" flower, according to STEVENS, is variable; from just above the ovary to close to the corolla opening. Each anther is topped by a pair of short awns. The ovary is similar in young buds of the two types of flower and, according to STEVENS, continues to be so up to the time of pollination and fertilization. There are two placentae per locule arising in the upper half (fig. 2*a*); these are axile below but become parietal at about the midpoint of their attachment (fig. 2*b*). These placentae are relatively small, projecting both upward and downward. Approximately one hundred small ovules are borne on all surfaces toward the outside of these placentae. The style springs from the base of the deep depression and, in the "pistillate" flower, is long and expands gradually upward to the enlarged and lobed stigma. In the "staminate" flower, on the other hand, the style is short, shows no expansion, and terminates in a lobed but unexpanded stigma. The surface of the ovary, in the "pistillate" flowers particularly, bears a great many hairs similar to those on the corolla.

Harrimanella stelleriana (Pall.) Coville. (*Andromeda stelleriana* Pall., *Erica stelleriana* [Pall.] Willd., *Andromeda em-*

petrifolia Mert., *Bryanthus stelleri* [Pall.] D. Don, *Mensiesia stelleriana* [Pall.] Fisch., *Cassiope stelleriana* [Pall.] DC.).—This is similar in certain respects to *Cassiope hypnoides*, but with a few differences evident. The parts are not quite so strongly reflexed; the sepals are diverged from one another from the base, with a faint suggestion of quincuncial aestivation in the old bud or flower which would probably be realized in a younger bud. The corolla is similar but with somewhat longer free lobes. Bases of the filaments are small, expand considerably, then taper to the anther. There may be somewhat fewer ovules per locule, sixty to seventy, but they are similarly placed on similar placentae. The style becomes very broad above the ovary and tapers to the narrow stigma. The nectariferous tissue is peculiar; it arises from the receptacle around the narrow bases of the stamens and is very highly lobed, extending down into the petal pockets and upward around the filaments, particularly between them.

Leucothoe axillaris (Lam.) D. Don. (*Andromeda axillaris* Lam., *Lyonia axillaris* [Lam.] K. Koch, *Leucothoe platyphylla* Small) (figs. 17, 44–53).—There are two small bracts on the pedicel just below the flower. They are essentially opposite each other and placed at right angles to the inflorescence axis. The relatively large sepals are imbricate. The corolla is long and narrow but urceolate, with only short free limbs which are recurved. At the base it is somewhat induplicate. The filaments, with a slightly swollen area near the base, become flattened laterally and are short, with the anthers located just above the ovary. There are no spurs or awns, but the relatively large lobe of connective tissue develops an area of disintegrating cells which breaks open in the older buds and flowers. The ovary may be slightly depressed, but occasionally it tapers upward into the style (fig. 17), which is essentially straight and terminates in an expanded and lobed stigma. The massive axile placentae arise near the top of the locules and have a large downward lobe close to the outer carpel wall. The twenty to thirty fairly large ovules per locule are borne on the lateral and inner surfaces of the placentae. The split of the central tissues connecting the locules with the stylar canal only barely affects the upper portions of the placentae. The nectary occurs at the base of the ovary, projects downward in ten short free lobes, and continues upward on the ovary wall in five major regions which, in this species, are opposite the petalad stamens.

Leucothoe catesbaei (Walt.) A. Gray. (*Andromeda catesbaei* Walt., *Andromeda spinulosa* Pursh, *Leucothoe spinulosa* [Pursh] G. Don, *Lyonia catesbaei* [Walt.] K. Koch).—This species of *Leucothoe* also has two small bracts. The corolla, fairly long and urceolate, has only short free limbs which are imbricate in the bud and may be recurved in the flower. The stamens are similar to those of *L. axillaris*, though the disintegrating area is not so extensive. The style, slightly enlarged above the ovary, terminates in an expanded and lobed stigma. The placentation and placental characters are also similar to those of *L. axillaris*. The nectariferous tissue is not very extensive and consists mainly of bulges between the filaments located on the ovary base.

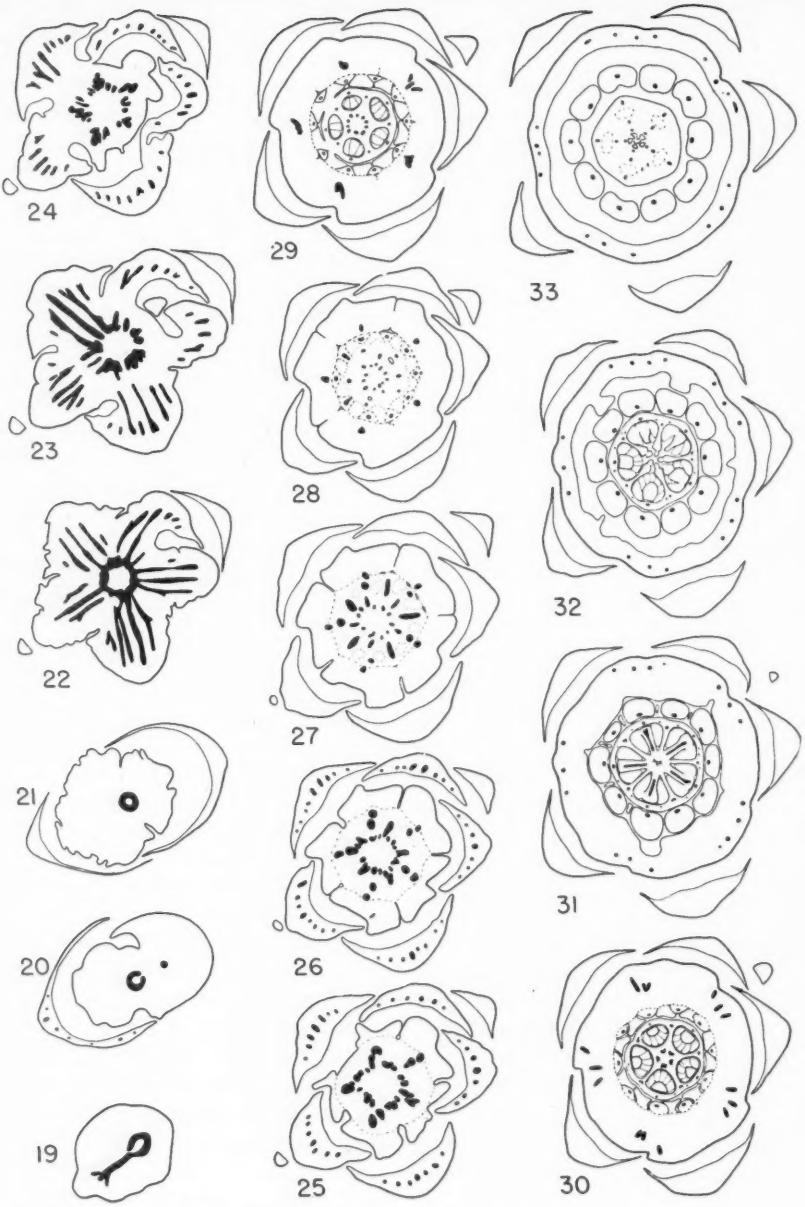
Leucothoe racemosa (L.) A. Gray. (*Andromeda racemosa* L., *Eubotrys racemosa* [L.] Nutt.).—Bracts, sepals, and corolla are similar to those of *L. catesbaei* except that the aestivation of both sepals and free petal lobes is quincuncial. The filaments are flattened above the swollen base and place the anthers at about the

midpoint of the flower. The projecting tissues of the connective above the filament-anther junction contain disintegrating areas. These disintegrate in the older bud, and sterile tissues on either side of them continue upward in four long awnlike projections (see fig. 6 of *L. recurva*). The exact nature of these projections is still to be determined, i.e., whether they are late-developing awns or merely pieces of a much elongated anther wall. The ovary is much as in *L. catesbaei*, but the split in the central tissues does not extend to the locules. The stigma is not enlarged. Nectariferous tissue forms part of the base of the ovary wall but has short free lobes extending up between the stamen filaments.

Leucothoe recurva (Buckl.) A. Gray. (*Andromeda recurva* Buckl., *Eubotrys recurva* [Buckl.] Britton) (figs. 6, 19–33).—In most respects this is similar to *L. racemosa* with a few exceptions. The free limbs of the corolla are very strongly recurved so that it appears only vaguely urceolate. The stylar canal does become continuous with the locules. The style is quite massive but narrows below the slightly expanded stigma. The nectary consists of ten lobes arising from the receptacle and extending upward between the stamen filaments.

Lyonia ligustrina (L.) DC. (*Vaccinium ligustrinum* L., *Andromeda racemosa*, Lam., *Andromeda paniculata* Ait., *Andromeda paniculata nudiflora* Michx., *Andromeda ligustrina* [L.] Muhl., *Lyonia paniculata* [Ait.] Nutt., *Lyonia salicifolia* Wats., *Lyonia capreaefolia* Wats., *Lyonia multiflora* Wats., *Lyonia ligustrina* var. *capreaefolia* and *salicifolia* DC., *Lyonia parabolica* K. Koch, *Xolisma ligustrina* [L.] Britton, *Arsenococcus ligustrinus* [L.] Small) (fig. 16).—The sepals are undiverged laterally in a relatively heavy basal area; the free limbs, thin and tapering rapidly to a point, are valvate in aestivation. The corolla is very short, broad, and extremely urceolate, with only short free ends which are valvate. The filaments are extremely small at the base but become greatly expanded above and then taper to the anther. They are actually long but form a huge horizontal **S** in the older buds and flowers, so that the anthers are against the style at about its midpoint. Very short, downward and laterally projecting spurs, formed largely of tissues which disintegrate, arise from the filament margins below its point of attachment with the anther (see fig. 7 of *L. mariana*, which is similar). The disintegrating areas continue upward. These spurs are observable only on young filaments, as they have disintegrated completely on mature ones, and only the ragged lateral margins of the filaments remain. The fairly massive placenta, arising in the upper half of the locule, and having a downward lobe, bears thirty-five to forty-five medium-sized ovules on the outer and lower surfaces. The style has a swollen area in the middle, then tapers to the unexpanded stigma. The lower third of the ovary wall is much enlarged with nectariferous tissue, ten bulges at the very base, then becoming five above.

Lyonia mariana (L.) D. Don. (*Andromeda mariana* L., *Andromeda pulchella* Salisb., *Leucothoe mariana* [L.] DC., *Pieris mariana* [L.] Benth. & Hook., *Neopieris mariana* [L.] Britton) (figs. 7, 18).—The sepals are diverged from one another at the base but have a considerable downward lobe and in this region are "joined" to one another by their inner surfaces, leaving the margins free. The free lobes above are quite massive and reduplicate in aestivation. The corolla has only short free limbs and may show in the upper regions a joining some-



FIGS. 19–33.—*Leucothoe recurva*, bud, ×16. Diagrams of serial cross sections of bud from pedicel to top of ovary.

what similar to that of the sepals—by the inner surfaces with the margins free outward. The corolla is large, long, and bell-shaped. Stamens correspond very closely to those described for *L. ligustrina*, and the pistil is also quite similar except that the placentae are more massive and bear in the neighborhood of two hundred small ovules per locule. The nectariferous tissue is also similar.

Neopieris nitida (Bartr.) Britton. (*Andromeda lucida* Lam., *Andromeda nitida* Bartr., *Andromeda coriacea* Ait., *Andromeda myrtifolia* Salisb., *Andromeda marginata* Veillard, *Lyonia marginata* [Veillard] D. Don, *Leucothoe coriacea* [Ait.] DC., *Leucothoe marginata* [Veillard] Spach., *Andromeda lacustris* C. Wright, *Pieris nitida* [Bartr.] Benth. & Hook., *Andromeda obovata* Raf., *Desmothamnus nitidus* [Bartr.] Small, *Desmothamnus lucidus* [Lam.] Small).—The sepals are imbricate in aestivation and quite large. The rather long, somewhat urceolate corolla shows quincuncial aestivation of the short free limbs in the bud. Stamens are characterized by large spurs which arise from the lateral edges of the filament and extend downward and then inward around the filament. Each possesses a large outer area of early disintegrating tissue (figs. 9, 10) which continues onto the filament and connective but terminates below the pair of awns which arises at each anther top. The filament becomes horizontally S-shaped in the flower. The fairly massive placenta has a large upward lobe which becomes double. The approximately sixty ovules per locule are fairly small and are borne on the outer surface of the placenta. No well-defined nectary is present, but there appears to be a little nectariferous tissue at the ovary base.

Oxydendrum arboreum (L.) DC. (*Andromeda arborea* L., *Lyonia arborea* [L.] D. Don) (figs. 4, 11, 12).—There are two small bracts arising from the pedicel in the same plane, but one is considerably higher than the other. The massive, but short, sepals show valvate aestivation and are not reflexed. Petals are free only at the tip, where they show quincuncial aestivation in the bud. The corolla is long and urceolate. The filaments become considerably enlarged at the base and taper to the anthers, which are located just above the ovary. The outer edge of the connective near the anther top shows a region of disintegrating cells which continues upward into the rather short awn. The medium-sized placenta arises from a large area and extends upward. The forty to fifty rather large ovules per locule are borne on the outer, outer lateral, and upper surfaces of the placenta. The long, straight style terminates in a nonexpanded stigma. Nectariferous tissue is rather late in developing and is constituted merely of somewhat enlarged areas on the base of the ovary wall. Hairs occur on the corolla, ovary, and to a lesser extent on the calyx of the young buds. One flower was observed which showed a reduced number in several whorls: there were five sepals, four petals, nine stamens (five outer and four inner), and four carpels.

Pieris floribunda (Pursh) Benth. & Hook. (*Andromeda floribunda* Pursh, *Leucothoe floribunda* [Pursh] D. Don, *Zenobia floribunda* [Pursh] DC., *Portuna floribunda* [Pursh] Nutt., *Andromeda montana* Buckl.).—The heavy sepals show valvate aestivation. The strongly urceolate corolla has large lobes extending between the sepals at its base, and the short free tips of the petals, imbricate in the bud, become slightly recurved in the flower. The filaments, particularly of sepalad stamens, are undiverged from the corolla for a short distance in the

base of the flower. They are small here but expand considerably above, then taper to the anthers. Spurs extend downward and outward from the outer corners of the filament or from the connective just above the juncture of anther and filament. In lobes of the connective above this area there are granular pouches which continue up into two short awns (see figs. 5 and 8 of *Ampelothamnus*, which is similar). The filaments become slightly S-shaped (vertically) in the flower. The medium-sized placentae arise in the upper half of the locules, and each has a downward lobe which bears fifteen to twenty fairly large ovules on the outer, lateral, and basal surfaces. The nectary consists of ten lobes of tissue extending up between the filaments. It arises from the receptacle, not the ovary wall. A few flowers were observed with six carpels.

Pieris japonica (Thunb.) D. Don. (*Andromeda japonica* Thunb.) (figs. 34–43).—The flower of this species is similar to that of *P. floribunda*. The pouching of the base of the corolla is not so great, and the stamens are diverged from the corolla. The spurs are larger, but otherwise stamen structure is similar. Placentation does not really become parietal in the locule top. The nectary lobes arise from the ovary base, which may have some additional nectariferous tissue. One flower was found which had four sepals (one extra large), four petals, nine stamens (four outer and five inner), and four carpels.

Xolisma fruticosa (Michx.) Nash. (*Andromeda ferruginea fruticosa* Michx., *Andromeda ferruginea* Pursh, not Walt., *Lyonia feruginea* [Pursh] Nutt.).—The flower of this species is in many ways quite similar to that of *Lyonia ligustrina*. The sepals are undiverged from one another for a short distance, the free limbs valvate. The corolla is very similar in shape, the free limbs imbricate in the bud. Stamens show considerable similarity. There are very short free spurs, and the filaments show regions of disintegrating tissue on the margins below their junction with the anthers. These regions continue in the connectives and the short awns, which are two per anther. The ovary characters show considerable similarity. The nectary consists of enlargements of the base of the ovary wall between the filaments. The biggest difference is the presence of a thick mat of hairs and scales on the receptacle, calyx, outer surface of the corolla, and ovary wall. One flower was observed which had five sepals (one extra large), six petals, twelve stamens, and five carpels (one of these also extra large).

Vascular anatomy

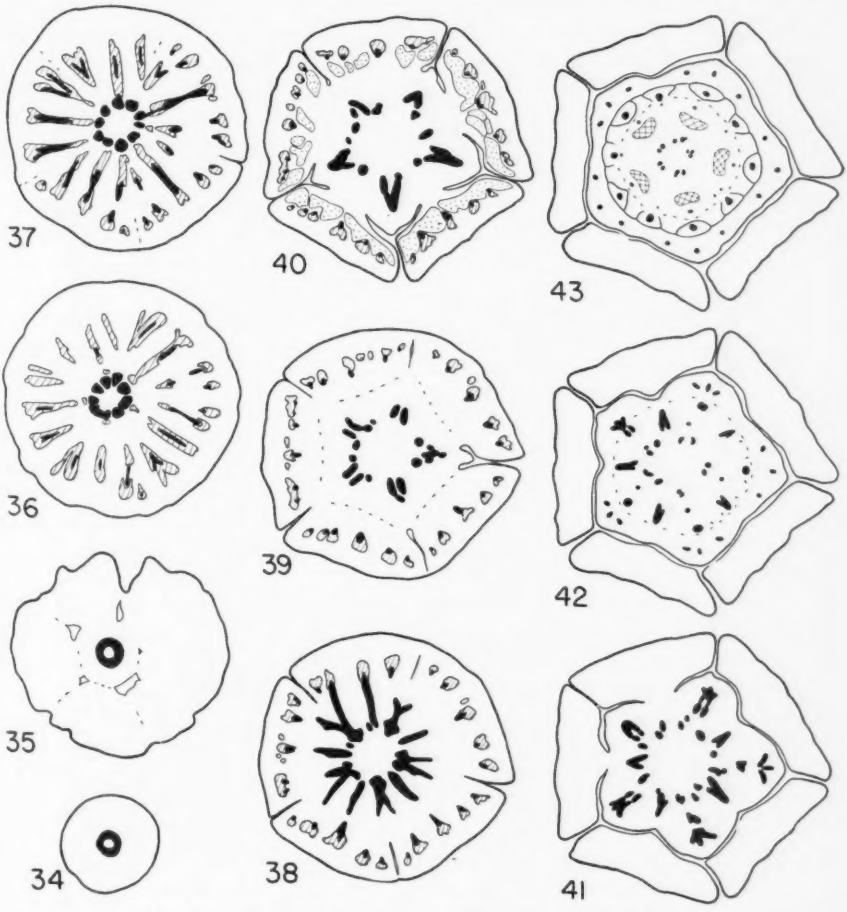
General.—The vascular tissues form a continuous cylinder in the pedicel (figs. 21, 34, 44, 54). This is broken by the traces to bracts when they occur (figs. 19, 20). The bract supply may vary: (*a*) a single small trace which remains undivided in the bract; (*b*) a single trace which divides to form three or many bundles in the bract (figs. 19, 20); (*c*) a single large trace which splits to three while still in the cortex and then redivides still more in the bract itself; or (*d*) five or more traces which arise independently, each leaving its own gap, and each subdividing in the bract, which thus has a great many bundles. Above the bract traces the gaps close, and a complete vascular ring enters the receptacle, where it normally broadens before giving rise to traces to the floral organs.

As many as seven whorls of traces, of five to fifteen bundles per whorl, may arise from the receptacular vascular tissues. These are, in acropetal order, the

vascular traces to the sepals, petals, petalad stamens, sepalad stamens, and dorsal, septal (or lateral), and ventral regions of the carpels. Certain of these whorls may arise together, so that the total number of whorls of bundles departing from the central cylinder might be as few as three (though none of the species studied has as few as this). The only whorls which may contain more than five bundles are the sepal and septal carpel whorls.

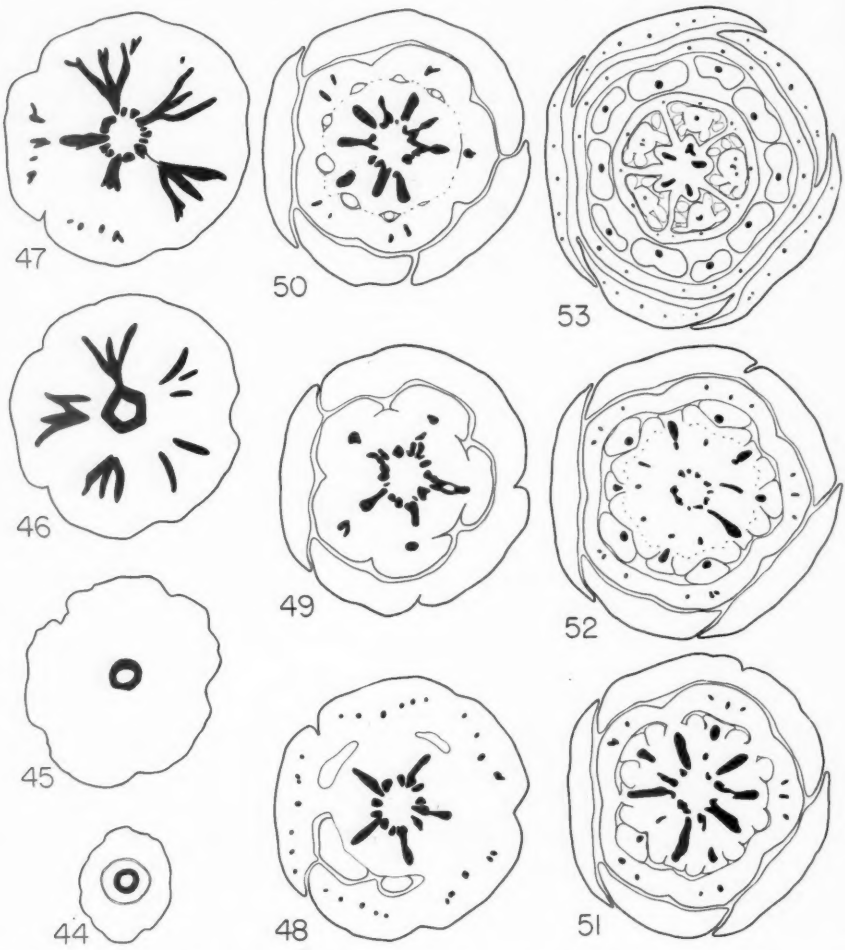
Reflexion of the floral organs is evident in the course of the vascular bundles in several of the species. In certain of them the traces have a strong downward curvature starting close to their origin, before curving upward. The curvature is usually greatest in the sepals and least in the carpels (figs. 13, 15); the reverse condition is found in one species (fig. 14).

The vascular supply of the sepals is of several sorts. (*a*) Three traces, each aris-



FIGS. 34–43.—*Pieris japonica*, bud, ×18. Diagrams of serial cross sections of bud from pedicel to base of locules. Crosshatched areas are base of locules; stippled areas, air space; single hatched areas, fiber tracts.

ing independently from the central cylinder, supply each sepal, thus leaving fifteen gaps in the vascular ring (figs. 22–24). (*b*) The base of each sepal receives three traces. The median trace arises independently from the central cylinder; the laterals of two adjacent sepals, however, arise as a common bundle between the median traces and in the plane of the petal bundles. This bundle splits radially shortly after its origin, half going to each sepal. This type of supply leaves ten gaps in the receptacular ring (figs. 36–38). Occasionally the joint lateral sepal traces arise so close to the petal traces that they may be considered downward branches of the latter, diverging from them at their points of origin (fig. 14). (*c*) For each sepal a single trace leaves the central cylinder but immediately separates radi-



FIGS. 44–53.—*Leucothoe axillaris*, bud, ×20. Diagrams of serial cross sections from pedicel at bottom of bud to base of ovary.

ally to three strands, which enter the base of the sepal and may there redivide (figs. 46, 47). Occasionally one of the lateral bundles may be derived from the adjacent petal bundle rather than from the median sepal trace. This type of supply leaves five gaps in the central vascular cylinder. (*d*) For each sepal a single trace leaves the central cylinder and remains undivided or forms three to five bundles in the outer portions of the sepal (figs. 54–57).

There is overlap in some species between a sepal supply of type *a* and of type *b*, so that some sepals may show three independent traces, and others, in the same flower, may have one lateral trace arising conjointly with the lateral from the adjacent sepal. There is also some overlap between type *a* and type *c* above, though perhaps not so frequently. When this occurs, two traces arise independently, and the third is derived from the median trace. In types *a* and *b* the lateral traces frequently branch while still passing out to the base of the sepals, so that the sepal base has five to seven traces, and this number may increase at a higher level in the sepal. Only three species have fibers accompanying the vascular bundles in the sepals.

The vascular supply to each petal is basically a single trace, the five traces alternating with the plane of the median sepal traces (figs. 25–26, 48). In a few cases where lateral traces of adjacent sepals arise together and the gap is not closed by the level of the petal trace, the single petal trace arises as two strands, one from either side of the gap, which come together close to their point of origin (figs. 39, 40). Petal traces may continue as single bundles throughout the entire length of the corolla or may divide in the corolla, but more frequently one divides to three strands at the base of a petal (figs. 29, 30, 41, 51), and these three bundles traverse the corolla; occasionally the division may occur while still in the receptacle. The numbers may become greater than three in the corolla.

The whorl of bundles to the petalad stamens, if separate from the petal whorl, arises close above and in the same plane as the petal whorl (figs. 11, 12, 14). As a consequence, the single trace to each stamen usually arises as a double strand from either side of the petal gap, the two coming together very soon in their outward course (figs. 49, 50). In several species, however, traces to the petalad stamens do not have an origin independent of the petal traces, but the two arise together. They become separate by a tangential division of the common trace somewhere in its outward course (figs. 13, 15, 16, 41, 57, 58). Each bundle remains single through the filament and into the anther where, just before its termination, it has a sharp downward bend in the connective (figs. 12, 15) (except in those few species in which the anther approaches the horizontal where the bend approaches a right angle).

Traces to the sepalad stamens, arising in the plane of the median sepal traces, most commonly are derived independently from the central cylinder. Usually the sepal gap has closed before their origin (figs. 26, 50, 51), but, in a few cases where it has not, the single trace has a double origin (figs. 41, 42) as described above for the petal or petalad stamen. In a few instances the sepalad stamen trace has a common origin with the median sepal trace, separating from it tangentially during the outward course of the common bundle (figs. 15, 57–60). There is a terminal bend in the bundle as described for the petalad stamen.

The dorsal carpel bundles are small and arise in the plane of the petals and

petalad stamens. They may have an independent origin (figs. 27, 42, 51, 60, 61), from the median area if the gap has closed, or, if the gap has not closed, usually from one side of the gap, though occasionally from both. Sometimes they have a common origin with the petalad stamen bundles irrespective of whether the origin of the latter is independent or in common with the petal traces. In these cases it separates tangentially from the common strand in its outward course, usually after the separation of petal and petalad stamen bundles if the strand is a "triple" one. The dorsal carpel bundle normally passes undivided through the carpel wall, turns under the depression at the top of the ovary, and enters the style, where it has a position at the end of an angle of the stylar canal (figs. 1*d*, 11, 13–16, 18, 33). It continues through the style, occasionally dividing below the stigma, and fades out in the latter. If the stigma is strongly lobed, the bundles may "change" position so as to enter the lobes (which correspond to the septal areas).

The septal, or lateral, carpel bundles, which are also small, arise in the plane of the median sepal traces and the sepalad stamens. In a few species they arise independently (figs. 11, 12, 28, 61), but in most they have a common origin with the sepalad stamen bundles from which they diverge tangentially usually well out in the course of the common bundle (figs. 14, 17, 52). Occasionally more than one bundle arises, so that there may be as many as ten, or occasionally more, septal bundles at the base of the ovary (fig. 61). In many species they divide in their upward course, the small branches supplying the ovary wall between the septa and the dorsal carpel bundles (figs. 2, 31, 32). The main bundle(s) passes upward in the outer wall in the region of the septum to the top of the ovary. Here they may fade out or pass over the top of the ovary toward the depression. They fade out without reaching the base of the depression or entering the style, however (fig. 1*d*). In one species these traces remain well within the septa for a considerable distance at the base of the carpels before passing out to the outer ovary wall (fig. 14).

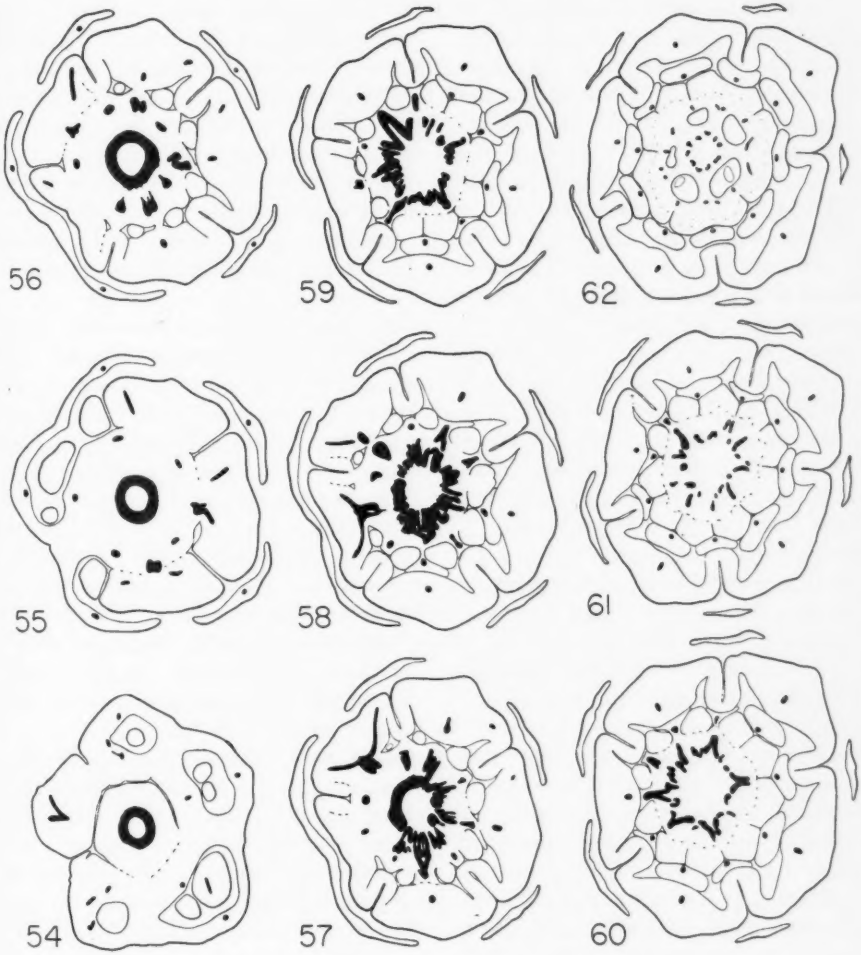
The vascular tissue remaining in the center constitutes the ventral carpel supply and in most species turns quite sharply inward and then upward (figs. 12, 18). It may be in the form of from five to fifteen small bundles, usually about ten, or an essentially continuous ring of vascular tissue (figs. 29, 43, 52, 62). Further upward rarely ten bundles are found, but normally there are five, which, in all except two species, correspond to the five locules, and thus each is the total ventral supply of a single carpel (figs. 1*a*, 30, 53). In the two species they correspond to the septa and so are constituted of half of the ventral supply of two adjacent carpels (figs. 2). This, as might be expected, is associated with a placentation which is parietal for a greater distance down from the top of the ovary than in the other species. These ventral bundles turn out into the placentae, where they bend and/or branch to supply the particular lobing of the placentae and thus the ovules.

The nectariferous tissue apparently receives no vascular supply except in *Epigaea* (and perhaps *Enkianthus*), where several small bundles arise from "excess" vascular tissue derived from both petalad and sepalad stamen traces.

INDIVIDUAL SPECIES.—*Ampelothamnus phyllireifolius*.—The sepal supply is of the three-trace, fifteen-gap type but with occasional sepals having adjacent laterals arising together. These three

traces branch so that several bundles traverse the sepals. Fiber tracts appear in the receptacle base, move into the sepals outside the traces, and form a heavy fibrous plate dorsal to the bundles through the entire length of the sepals. The petal traces have a double origin, form three strands in the base of the petals, and divide again farther up. The petalad stamen traces, independent of the petal traces and double at their origin, give rise to the dorsal carpel traces. The sepalad stamen traces are also independent, double at their origin, and give rise to the septal carpel strands.

Andromeda glaucophylla (figs. 15, 54–62).—The reflexed supply to each sepal is a single bundle throughout, while the



FIGS. 54–62.—*Andromeda glaucophylla*, bud, ×15. Diagrams of serial cross sections from bottom of bud to base of locules.

petal strand, also reflexed, forms three in the base of the corolla and occasionally five above. The trace to the petalad stamen arises from the petal bundle in the down curve of that trace, and similarly the trace to the sepalad stamen arises from the sepal bundle near the top of the curve. The dorsal carpel traces arise as a single strand from one side of the petal gap or occasionally have a double origin from both sides of the gap. Two septal traces arise, one on either side of a sepal-sepalad stamen gap; these may occasionally fuse but frequently remain independent.

Andromeda polifolia.—Definite similarities exist between this species and *A. glaucophylla*. The sepal traces may form three to five weak bundles above the base of the sepal. The petal and petalad stamen supply is similar except that the two separate at the top of the curve. The sepalad stamen trace in this species, however, has an independent origin and usually has the double supply—from both sides of the sepal gap. The dorsal carpel traces are similar, but the sepalad traces are single and normally arise from one side of the gap, though occasionally having a double origin.

Cassiope hypnoides.—The strongly reflexed sepal supply arises as a single trace but almost immediately forms three, all of which are quite small. The petal trace, also reflexed, is single but becomes three or occasionally more above the base of the corolla. The petalad stamen trace arises from the petal trace near the top of the downward curve, while the independently arising sepalad stamen trace usually has a double origin. The dorsal carpel trace arises from the petalad stamen trace, and the septal traces arise from the sepalad stamen traces.

Cassiope lycopodioides.—The supply to each sepal is a single, strongly reflexed trace which forms three or occasionally five small bundles above the base. The petal and stamen supplies are similar to those in *C. hypnoides*, but the dorsal and septal carpel supplies both arise independently.

Cassiope mertensiana (fig. 13).—The traces in this species are more strongly reflexed than in any other. The single trace entering a sepal is undivided. The petal traces are similar to those of the other species of *Cassiope*. The petalad stamen trace arises near the bottom of the downward curve of the petal trace with which it has a common origin, while the sepalad stamen trace arises near the top of the curve of its corresponding sepal trace and follows it in the downward curve. The dorsal and septal carpel traces are essentially like those in *C. lycopodioides*.

Chamaedaphne calyculata (fig. 1).—Each bract trace arises as a single large strand which almost immediately divides to three, these in turn dividing so that there are many bundles in each bract. The sepal supply is of the three-trace, ten-gap type, and the traces show essentially no reflexion. The petal bundle divides only well up in the corolla. The petalad stamen supply is from the petal bundles; the sepalad stamen supply is independent in origin. The dorsal carpel traces are independent in origin, while the septal traces are derived from the sepalad stamen bundles.

Enkianthus cernuus (fig. 14).—The sepal supply is of the three-trace, ten-gap type but is peculiar in that the three traces do not arise at the same level. The median traces arise at the very base of the receptacle or upper pedicel and move outward very gradually. The lateral traces arise at a much higher level and pass outward almost at right angles to the central cylinder. The joint lateral

bundles depart so close to the petal traces that they might be considered to be branches from them at their point of origin. The petal bundle is single to the base of the corolla; what happens in the corolla itself is not known. The petalad stamen traces are independent but double in origin and slightly reflexed. The sepalad stamen traces turn quite sharply downward, and the septal traces arise from these on the downward curve. The dorsal traces are independent in origin and very strongly reflexed. The septal traces are located in the septa for a considerable distance before reaching the outer carpel wall; they branch repeatedly. The five ventral carpel bundles which become organized are here located in the plane of the septa rather than that of the locules of the carpels.

Epigaea repens (fig. 2).—The one lower bract has a single trace dividing to three, while the upper ones are much more massive and somewhat variable in their supply. As few as five, or rarely three, and as high as nine traces, with their associated gaps, have been seen, these traces redividing so that the number of traces in a bract may be in the neighborhood of twenty. The sepal supply is of the three-trace, ten-gap type, the laterals, however, arising so close to the petal bundles as perhaps to be considered branches from these traces at their point of origin. Occasionally adjacent laterals may have an independent origin, thus overlapping a little with the fifteen-gap type. Petal traces branch to three very early, and, although they do not redivide in the corolla tube, they do in the free limb. The petalad stamen and dorsal carpel traces have an origin in common with the petal traces. First the petal and stamen supplies separate, then the stamen and carpel portions. The sepalad stamen traces arise independently of the sepal traces but in common with the septal traces; the latter diverge from the common bundle in the base of the ovary. In the "pistillate" flowers where the functional stamens are replaced by rather small staminodes, all the vascular tissue of the common bundle does not enter the staminodes or carpel traces, but an "excess" is left in the base of the ovary wall. This breaks up into several small bundles some of which turn outward and end blindly in the region of nectariferous tissue. The septal bundles divide freely. The dorsal bundles "move" in the upper part of the style to enter the large lobes. The ventral carpel supply is also peculiar in that the five bundles which eventually form are in the planes of the septa, not of the locules.

Harrimanella stelleriana.—Though not so strongly reflexed, the sepal and petal supply is similar to that in *Cassiope mertensiana*. The petalad stamens, however, have traces arising independently, though so close to the petal traces as to be double in their origin. The origin of the sepalad traces is similar to this, though alternating in position. The dorsal carpel traces are similar to those of *C. lycopodioides*, the septal to *C. hypnoides*.

Leucothoe axillaris (figs. 17, 44–53).—The two bracts each receive a single trace which branches in the bract itself to form several bundles. The supply for each sepal is slightly reflexed and arises as a single trace, immediately forms three and, in the sepal itself, redivides to form occasionally as many as nine bundles. The petal strand remains single through most of its length, occasionally giving rise to one or two branches high in the corolla. The petalad stamen trace arises from both sides of the petal gap, or occasionally from one side only, but the

sepal gap has closed before the departure of the sepalad stamen traces so that they are single to their very base. Dorsal carpel traces may arise from one side of the gap only or from both, while the septal traces diverge from the sepalad stamen traces. The ventral supply is somewhat unusual, as it first forms five bundles in the planes of the septa which send small branches into the septa, then reforms five different bundles in the plane of the locules, and these pass into the placentae from this more usual position.

Leucothoe catesbaei.—The bract supply and the sepal traces are as in *L. axillaris*. The latter, however, do not redivide so much in the sepal itself. Petal traces, on the other hand, regularly divide to give three at the base of the corolla. Stamen traces and outer carpel traces are also similar, the petalad stamen and dorsal carpel traces, however, regularly having a double origin. Ventral bundles are normal.

Leucothoe racemosa.—The bracts are supplied as in *L. axillaris*. The sepal supply, however, is not reflexed and is of the three-trace, fifteen-gap type, the three traces normally giving seven or more bundles in the sepal base. The petal regularly becomes three-veined at the base of the corolla, and the vascular supplies to the petalad stamens, which arise close above the petal traces, have a double origin. The sepal gap is normally closed before the departure of the sepalad stamen traces, but occasionally they, too, have a double origin. Dorsal and septal carpel traces normally arise independently, but both may occasionally be derived from their corresponding stamen bundles.

Leucothoe recurva (figs. 19–33).—The vascular system here is similar in most respects to that in *L. racemosa*. Petal and petalad stamen traces arise at almost the same level rather than completely independently, and the petal trace does not divide at quite so low a level in the corolla. Dorsal and septal traces regularly have an independent origin.

Lyonia ligustrina (fig. 16).—The sepal bases receive three slightly reflexed traces, but their origin is various, usually showing the five-gap, though sometimes the ten-gap or the fifteen-gap, type. Usually in any one flower there is some combination of these; for example, one sepal may show the median and one lateral trace arising together, the third arising independently or in common with the lateral from the adjacent sepal, etc. Farther out the sepals show five bundles. The petal traces form three each in the base of the corolla and close to their origin give rise to the petalad stamen traces. Sepalad stamen traces have an independent origin as do also the dorsal and septal carpel bundles.

Lyonia mariana (fig. 18).—The sepal supply, which is fairly strongly reflexed, is variable as in *L. ligustrina*, although usually it is only of the five-gap or ten-gap type or some combination of these, rather than including any laterals which are completely independent in origin. An occasional lateral arises from the adjacent petal trace. Further division results in seven or nine traces per sepal. Petal and stamen supplies are closely similar except that the petal traces divide to three close to their origin—that is, before the corolla base is reached. Dorsal and septal carpel traces arise from their corresponding stamen traces.

Neopieris nitida.—Traces here show no reflexion and tend slightly upward. The sepal supply is most frequently of the three-trace, ten-gap type, but there are frequent variations toward the five- or fifteen-gap types—that is, an occasional lateral arises from the median

trace or arises independently from the receptacular vascular cylinder. Five bundles are present well out in the sepal. Petal traces divide to three just below the base of the corolla. Petalad stamen traces arise close above the petal traces from both sides of the petal gaps. The sepalad stamen supply, also having an independent origin, is, however, only occasionally double at its base. Dorsal carpel traces are also double at their origin, but septal traces are derived from the sepalad stamen traces.

Oxydendrum arboreum (figs. 11, 12).—The receptacle here is more elongated than in any of the other species studied, so that all traces have an independent origin. The elongation becomes increased with age. None of the traces shows a reflexion; all have a definite upward trend. Each sepal receives three traces, the origin normally of the ten-gap type, though occasionally it may be of the five-gap, or rarely of the fifteen-gap, type. These three give rise to as many as seven in the sepal base. The petal trace remains single for a considerable distance but does eventually divide to three in the corolla. The petal gaps have closed before the departure of the petalad stamen traces, thus giving the only example in the species described in this paper of an independent petalad stamen supply which is not made up of strands derived from both sides of the petal gap. The sepalad stamen traces alternate with these and show a similar mode of departure. Dorsal carpel traces depart above the petalad stamen gap. From one to three septal carpel bundles arise from the central cylinder and pass outward and upward in the carpel wall.

Pieris floribunda.—The sepal traces are usually a little reflexed in the flower though not in the bud. They are normally of the three-trace, fifteen-gap type, with occasional adjacent laterals arising together. The number of traces early increases to seven or more. Fiber strands accompany the sepal bundles from the receptacle through the entire length of the sepal. The petal traces form three in the base of the corolla. The petalad stamen traces depart from the petal traces, but the sepalad stamen supply is independent in origin. Dorsal carpel bundles have a common origin with the petal and petalad stamen bundles and diverge from the latter after their separation from the former. Septal carpel traces are derived from the sepalad stamen traces.

Pieris japonica (figs. 34–43).—This shows certain similarities to *P. floribunda* but also certain differences. The sepal supply, which is also accompanied by fiber tracts, is usually of the three-trace, ten-gap type, so that the petal traces, arising close above the joint sepal laterals, have a double origin. Three traces are formed at the very base of the corolla, and five bundles appear higher up. The stamen traces are similar except that the median sepal gaps have not closed at the point of departure of the sepalad stamen traces so that the latter receive their supply from both sides of the gap. The dorsal carpel traces arise independently from one side of the petal-petalad stamen gap, while the septal carpel traces depart from the sepalad stamen traces.

Xolisma fruticosa.—The sepal supply, which is slightly reflexed, is of the three-trace, ten-gap type. A few small, short branches may arise from these three traces. The petal traces, double at their origin, form three bundles in the corolla base. The petalad stamen supply arises jointly with the petal supply and diverges from it in its outward course. Sepalad stamen traces arise independently but before the closure of the median sepal

gaps, so that each has a double origin. Dorsal carpel traces are independent in origin, but the septal traces arise from the sepalad stamen bundles. As can be seen, this shows several similarities to *Lyonia ligustrina*.

Discussion

ORGANOGRAPHY OF ANDROMEDEAE COMPARED WITH OTHER GROUPS OF ERICALES.—A brief comparison of some of the main points of floral organography between the Andromedeae described in this paper and other tribes, subfamilies, or families of the Ericales will serve not only to emphasize the relationships within the order but also to point out some of the tribal characteristics. Various facts relating to the other groups are taken from the following sources: for essentially all groups—DRUDE (26), MATTHEWS and KNOX (38), HANCY (33), WARMING (47), and FERNALD (28); Pyroleae—COPELAND (22) and HENDERSON (34); Monotropoideae—COPELAND (14, 15, 17–20), DOYEL and GOSS (25), OLIVER (41), and HENDERSON (34); Ericoideae—BEIJERINCK (4); Empetraceae—MENTZ (39) and HAGERUP (31).

The characters of the Andromedeae listed earlier which can be compared readily are the following: pentamery, pentacycly, sympetaly, actinomorphy, hypogyny, introrse stamens in bud and flower, non-epipetalous stamens (except for one minor exception in *Pieris floribunda*), staminal appendages, hermaphroditism (except in *Epigaea*), various characters of the placentation and number of ovules, and the nectary. Variations occur both up and down with respect to numbers within a whorl, though the most common number is five. Some of the Monotropoideae, Ericoideae, Vaccinioideae, and an occasional member of the Rhododendroideae are tetramerous, while all whorls in *Empetrum*, and the carpels of *Clethra*, the Diapensiaceae, and certain Rhododendroideae are reduced to three. On the other hand, some species of Rhododendroideae are heptamerous with the carpel number even higher.

Most of the main groups of the Ericaceae proper are also pentacyclic with the outer whorl of stamens opposite the petals. Certain cycles, however, may be eliminated. In some of the Monotropoideae the calyx is reduced or lacking, and *Empetrum* is apetalous. More usually the flowers are tetracyclic by the loss of the petalad whorl of stamens. This is the case in a few of the Rhododendroideae, *Empetrum*, the Diapensiaceae, and the Epacridaceae.

On the whole members of the order are sympetalous, but polypetalous genera and species are found: the Pyroleae, some of the Monotropoideae, certain species of *Vaccinium*, occasional members of the Ericoideae, and the Clethraceae. Actinomorphy is also the rule, but some of the Pyroleae, some of the Rhododendroideae, and an occasional member of the Ericoideae are zygomorphic, though not strongly so.

Hypogyny is found in all groups except the Vaccinioideae which are epigynous. The ovary in *Bejaria* (Rhododendroideae) may be slightly imbedded, and that of *Chiogenes*, which has been variously classed in the Vaccinioideae and in the Gaultherieae, is reported to be quite deeply imbedded. The ovary in *Gaultheria* is superior but covered by a large, fleshy calyx.

In the Pyroleae, Arbuteae, and Clethraceae the anthers go through the early stages of development without bending inward and so would be considered extrorse, but at anthesis they invert, so that in the open flower their

position is introrse—the condition in the other groups. The stamens are only exceptionally epipetalous in the main groups of the Ericaceae but are regularly so in the Epacridaceae and Diapensiaceae. Staminal appendages are found in many species of the Arbuteae, Ericoideae, Vaccinioideae, Gaultherieae, and one genus of the Monotropoideae as well as in the Andromedeae, but not in the other groups.

The only regularly occurring example of unisexuality of the flowers is the Empetraceae, but *Empetrum* may be bisexual in certain localities. *Epigaea* appears to be in the process of becoming unisexual, for, although it may have both stamens and pistil present in some form, only one or the other functions.

In most of the groups the placentae are axile at the base and at least approach a parietal condition in the ovary top. The parietal condition is best developed in the Monotropoideae, and here and also in the Pyroleae the placenta is double in each locule. The ovule number is in general high, but low numbers are found in some of the Arbuteae, a few genera of Rhododendroideae, and the Epacridaceae.

Nectariferous tissue is generally present in those groups for which information was found, though not greatly developed in the epigynous forms—the Vaccinioideae, and in some of the Pyroleae.

Additional taxonomic characters supplement those considered briefly above to separate families, subfamilies, and tribes in any system of classification.

VASCULAR ANATOMY OF ANDROMEDEAE COMPARED WITH OTHER GROUPS OF ERICACEAE.—The details of vascular anatomy are less well known; they have been reported for a relatively limited number of species. The most thorough treatments of groups as a whole are those of COPELAND for the Rhododendroideae (**21**), Pyroleae (**22**), and Monotropoideae (**14, 15, 17–20**). HANCY (**33**) described the structure in a number of species belonging to several groups, while two papers deal with individual species: DOYEL and GOSS (**25**) on *Sarcodes* and BAKER (**3**) on *Vaccinium macrocarpon*.

HANCY described two species studied in the present paper: *Andromeda glaucophylla* and *Chamaedaphne calyculata*. She reported, for *Andromeda*, a whorl of five distinct calyx bundles, a whorl of combined petal and petalad stamen bundles, and a whorl with sepalad stamen bundles and carpel bundles (which occur in the planes of the septa and terminate without entering the style) arising at the same level. She said nothing of dorsal or ventral carpel bundles, although these are shown in the diagrams, made no mention of, nor showed, gaps in the vascular cylinder, and considered *Chamaedaphne* to have essentially the same structure as *Andromeda*. The present observations do not agree with these on several points. In *Andromeda* the sepalad stamen strands were found to have a common origin with the sepal bundles, and the septal carpel traces to be independent in origin. Both ventral and dorsal carpel strands are clearly present, the latter entering the style and ending at the level of the stigma. Gaps are associated with the departure of the various traces as described earlier. *Chamaedaphne* differs from *Andromeda* in several respects. The sepal supply is not restricted to a single strand, but each sepal receives three traces, each lateral arising conjointly with a lateral from the adjacent sepal so that a total of ten gaps occurs in the central vascular ring. In this species the sepalad stamen supply is independent in origin and does give rise to the septal

bundles. Again both dorsal and ventral carpel traces are present.

Three traces with three associated gaps is considered to be the primitive condition of the vascular supply to a sepal (**22, 27**). This is found in certain of the Andromedeae as reported and again in *Bejaria* of the Rhododendroideae, and occasionally in *Chimaphila* of the Pyroleae, though the latter may show only one or two gaps associated with its three traces. This condition of the reduction in the number of gaps has been seen in several species of the Andromedeae. In *Elliottia*, *Menziesia*, *Rhododendron* (used in a restricted sense omitting *Azalea*, *Azaleastrum*, and *Hymenanthes*), and *Ledum* a single trace departs but promptly forms three, a type of sepal supply also observed in several of the Andromedeae. In *Rhodothamnus*, *Dendrium*, *Kalmia*, *Phyllodoce*, *Daboecia*, *Cladothamnus*, and *Loiseleuria* of the Rhododendroideae, *Pyrola* and *Moneses* of the Pyroleae, and *Clethra* a single median sepal trace arises independently, and the laterals are supplied from the adjacent petal strands. This condition was seen only occasionally in two species of the Andromedeae, though it was approached in *Enkianthus* and *Epigaea*. The other Andromedeae, other Rhododendroideae, *Gaultheria procumbens*, several species of *Vaccinium* and *Gaylussacia*, and the Monotropoideae regularly have a single sepal trace which may or may not divide in the sepal itself.

The method of origin of the lateral sepal bundles in *Epigaea* and *Enkianthus* may give some evidence to help determine the answer to a question raised by COPELAND (**21**) with regard to the lateral sepal traces being derived from the adjacent petal bundles. "One may speculate as to whether it [the pattern of origin of lateral sepal traces] is established by a shifting of the sepal lateral bundles to the petal bundles, or alternatively, by the fusion of the petal bundles with sepal bundles which had been free of others, as in *Bejaria*." It is suggested that two types of reduction have possibly occurred phylogenetically in the basic three-trace, three-gap sepal. In one the origins of the three traces to one sepal have come closer and closer together, until the three depart as a common bundle, leaving a single gap in the cylinder. This bundle separates to its three component strands while still in the receptacle. In the other type of reduction the laterals of adjacent sepals have come closer together in their point of origin, until they arise as a single bundle in the plane of the petal. Each pair of laterals thus leaves a single gap, and each median sepal trace a single gap, so that a total of ten gaps results in the vascular tissue of the receptacle. If, then, the internode between the lateral sepals and the petal traces were to become so compacted that these traces arose together, a condition foreshadowed by *Epigaea* and *Enkianthus*, the lateral sepal traces would appear to be derived from the petal traces. Both lines of reduction could lead to the single-trace sepal, in the first by a nonseparation of the three traces close to their point of origin and in the latter by a nondivergence of the sepal supply from the petal strands.

If such were the course of phylogeny, *Chimaphila* would be the more primitive genus of the Pyroleae and *Bejaria* of the Rhododendroideae, and so they are considered by COPELAND. Applying this to the Andromedeae, *Leucothoe racemosa*, *L. recurva*, *Ampelothamnus*, and *Pieris floribunda* would be the more primitive, while on one line of reduction would be found *Leucothoe axillaris*, *L. catesbaei*, *Lyonia ligustrina*, and *L. ma-*

riana and on the other *Chamaedaphne*, *Pieris japonica*, and *Xolisma*, with *Epigaea* and *Enkianthus* almost to the point of having the lateral sepal strands derived from the petal bundles. *Neopieris* and *Oxydendrum* have not progressed far in reduction, as they occasionally show three gaps, and are not set on one line of reduction, though more frequently showing the characteristics of the second line, i.e., lateral sepals arising together. The species of *Andromeda*, *Cassiope* (except for *C. hypnoides*), and *Harrimanella* have reached the single-strand stage. The fact that in *C. hypnoides* the single strand divides early to three might suggest that the single-strand condition in the other species of *Cassiope* and in *Harrimanella* might have been derived along that line of reduction.

The petals in all forms that have been reported receive a single trace with the exception of *Chimaphila*, where COPELAND reported that the three bundles in the base of a petal may be accompanied by one to three gaps in the vascular ring, i.e., the petal receives one to three traces. In *Pyrola* and *Moneses* lateral bundles may be derived from adjacent sepal traces. In many forms the single trace will form three while still in the receptacle—for example, in *Elliottia*, *Menziesia*, *Rhododendron* (restricted), *Ledum*, many of the Monotropoideae where the median trace may become small or be lacking (*Newberrya*), and in certain of the Andromedeae such as *Ampelothamnus*, *Epigaea*, *Lyonia mariana*, *Neopieris*, and *Pieris japonica*. In others the trace may divide in the base of the corolla or higher up.

A single trace supplies each stamen and in all forms except *Daboecia* (21) shows a bend in the connective associated with the introrse position of the anther. In *Daboecia* the bend occurs, but there is also a strand which runs up in the connective above the bend.

Other groups may differ somewhat from the Andromedeae with respect to the carpel supply. The dorsal carpel bundles arise consistently in the plane of the petals, though at their origin they may be represented by very small bundles that are difficult to find, as in some of the Monotropoideae. In most species these strands form the stylar vascular tissue. No stylar bundles are found in *Pterospora*, which has no dorsal carpel strands. In *Monotropsis* branches of these bundles supply the nectary. Septal bundles are not so regular in their occurrence. They are found in *Chimaphila* in the plane of the sepals and continue into the style along with the dorsal strands. In the Rhododendroideae their origin is various and may in part be associated with a vascular supply to nectariferous tissue. They are only occasionally found in the Monotropoideae, and, although HANCY did not describe them in *Vaccinium*, BAKER reported, in *V. macrocarpon*, the presence of small bundles arising from the sepalad stamen traces, passing toward the style in the planes of the septa and apparently supplying the nectariferous tissue. Such a small bundle is shown, however, in HANCY's diagram of this species. Unpublished observations by the present author on *V. vacillans* show the regular occurrence of a bundle in this position. The ventral carpel bundles in the Pyroleae and Monotropoideae are associated with the septa—that is, are composed of half the vascular supply of two adjacent carpels—while in the Rhododendroideae they are associated with the locules of the carpels as in most of the Andromedeae. The condition in *Vaccinium* is difficult to determine from HANCY's report, but observations on *V. vacillans* show the ventral traces in that

species to be associated with the locules. The ventral bundles provide the placental and ovule supply, and in addition in *Pyrola*, *Moneses*, and *Sarcodes* they also continue into the style.

The degree of nondivergence among various strands is varied in the other groups just as it is in the Andromedeae, and the close origin of one trace above another—for example, in *Chimaphila* and *Monotropa*—is accompanied by a double origin of the traces—part from either side of the gaps formed by the lower whorl of traces, just as in certain species described earlier. In *Pyrola* the traces to petals, petalad stamens, and dorsal carpel areas may have a common or independent origin. In the Rhododendroideae the petal and petalad stamen supply is often common in origin as also in *Sarcodes*, *Allotropa*, and *Clethra*. There is less nondivergence between sepal and sepalad stamen just as was seen in the Andromedeae, but septal carpel bundles may frequently arise from their corresponding stamen traces. The greatest degree of nondivergence among strands to various floral organs is seen in the Vaccinioideae, where, in the extreme cases, the vascular ring of the pedicel breaks up into ten bundles, and from these ten all the different organs are supplied, the divergences occurring at various levels from the base of the ovary to the top.

"Excess" vascular tissue, apparently similar to that described in *Epigaea*, has been reported by COPELAND (15) in *Pityopus californica*. It is derived from the stamen bundles, and the small bundles into which it breaks up may either fade out or eventually supplement the regular carpel bundles. No mention is made of its supplying nectariferous tissue.

CLASSIFICATION OF, AND WITHIN, THE ERICALES.—The Ericales and its subgroups have been variously placed as indicated in the "Introduction." What characters have been considered of prime importance in classification systems? The taxonomic systems such as that of ENGLER and PRANTL, BESSEY (5), and HUTCHINSON (35) use several morphological and vegetative characters, but the primary character used is different in each case; for example, polypetaly versus sympetaly, hypogyny versus epigyny, and woody versus herbaceous, respectively, are the main criteria used for each of these systems. This results in a different placing of the order. BROWN's (8) study of nectary forms led him to derive the Ericaceae from the Theales, thus supporting HUTCHINSON's position of the order. ZIEGENSPECK's (48) position of the family, based on serology, shows no relationship to any other system of classification except that it tends to corroborate the alliance of the Empetraceae, a family occasionally placed in the Sapindales, with the Ericaceae. HALLIER's (32) placing of *Saurauia*, *Clematoclethra*, and *Actinidia* with *Clethra* in the Ericaceae, although LECHNER (36) separated the first three from the last, suggests a relationship with the Dilleniales. At the present time, however, with the knowledge of the various families of the Ericales incomplete and little detailed information available on the orders with which they have been allied, it is of more interest to consider the classification within the order itself.

HAGERUP's chromosome study (30) of a number of different species, including the Empetraceae, Clethraceae, Diapensiaceae, and Epacridaceae as well as the Ericaceae (including *Pyrola* and *Vaccinium*), has shown that a progressive series can be set up with six as a basic number. On morphological and anatomi-

cal grounds COPELAND (16) removed the Lennoaceae from the order, while a study of seed development (44) and other morphological characters (31) places the Empetraceae within the order, although the position of the Diapensiaceae remains somewhat in doubt.

Various lines of development have been set up within the Ericaceae proper based on different characters. For example, DRUDE (26), using several characters—including the singleness or doubleness of the placental tissue in a locule—found the Vaccinieae, Thibaudieae, Arbuteae, Gaultherieae, and Andromedeae more closely related to one another than to the Ericoideae or Rhododendroideae. MARTIN (37) considered the type of seed found in the Vacciniaceae as more primitive than that of the Ericaceae proper, while that in the Pyrolaceae and Monotropaceae is most advanced.

ARTOPOEUS (1) based a series on the method of anther dehiscence. There are five groups from the more primitive to the more advanced: (*a*) the pore opening results from the activity of an endothecium as seen in *Clethra;* (*b*) formation of long clefts with remnants of an endothecium as seen in *Rhodothamnus*, *Phyllodoce*, and to a certain extent in *Kalmia;* (*c*) dehiscence by dissolution tissue, with or without resorption tissue, as begun in *Kalmia* but well developed in *Rhododendron* and *Erica;* (*d*) dehiscence through the dissolution of the central tissue of long awns as found in the Vaccinioideae; and (*e*) clefts arising by unequal contraction of cells of a newly acquired exothecium, local in position in *Arbutus* and the entire length of the anther in *Loiseleuria* and the Epacridaceae. The Andromedeae and Gaultherieae are transitional between *Rhododendron* and *Vaccinium*. They have no resorption tissue such as that found in *Erica*. This intermediate position of the Andromedeae is in part corroborated by MATTHEWS and KNOX (38).

COPELAND (22), on the basis of his detailed morphological work, has decided that the *Pyrola* group of plants should be a tribe of the Arbutoideae closely related to the Andromedeae, while the *Monotropa* group consists of highly reduced forms best placed in a separate subfamily but derived from the Andromedeae independently of the Pyroleae. The present study of vascular anatomy in the Andromedeae will serve to supply the details of similarity between this group and the Pyroleae which were anticipated but not known by COPELAND. He maintains the Rhododendroideae as an independent subfamily (21).

Perhaps even more interesting, because it deals with species discussed in this paper, is COX's classification of the Arbutoideae (24) based primarily on wood anatomy. He considered the subfamily to be monophyletic and probably to have a common ancestry with the Rhododendroideae, a subfamily which he also considered to be monophyletic, with the possible exception of the Bejarieae (23). The Arbutoideae he subdivided into five tribes: the Cassiopeae, Gaultherieae, Andromedeae, Oxydendreae, and Arbuteae. Of particular interest are the three which include the species which have been studied morphologically in this paper. His tribe Cassiopeae includes *Enkianthus*, *Cassiope*, *Epigaea*, and *Agauria*, the Andromedeae—*Andromeda* (genera such as *Xolisma*, *Lyonia*, and *Pieris* are placed in *Andromeda* because they differ so little from one another, but *Andromeda* proper, i.e., *A. polifolia* or *A. glaucophylla*, was not studied), *Chamaedaphne*, and *Leucothoe*—and the Oxydendreae—*Oxydendrum*. In each tribe the more primitive genus

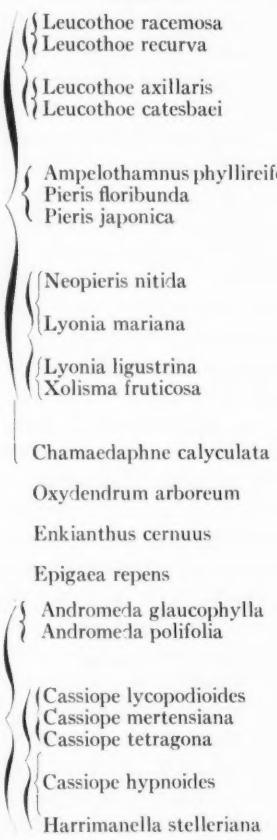
is given first, the more advanced last. As shown in the following discussion, a tentative arrangement of the genera based on the present study in part substantiates Cox's arrangement but in other respects differs rather sharply from it.

It is interesting that all the species studied have, with the exception of *Epigaea*, been placed at some time in the genus *Andromeda*. This can be noted from the synonymy for each species given in the section on organography as compiled from BRITTON and BROWN (**7**), SMALL (**45**), and BAILEY (**2**). This similarity is reflected in Cox's lumping of several genera into this genus. On the basis of the present study, however, *Andromeda* occupies a somewhat isolated position.

TENTATIVE CLASSIFICATION OF THE ANDROMEDEAE.—No single character of those considered, either of organography or of vascular anatomy, can be used in arranging a tentative grouping of the species studied. The first separation is based on the nature of the sepal supply. Other characters are used in addition to this for the further subdivision; those features to which somewhat greater importance is attached are: presence or absence of fibers with the sepal traces, position and character of the placenta, position of ventral carpel bundles, the reflexion of the floral parts and particularly of their vascular supplies, the joint origin of the vascular supply to different organs, and staminal appendages; and of less importance: various characters of the nectary, position of anther-filament junction, corolla shape, hairs, filament swellings and shape, stigma size, ovule number and size, and aestivation of sepals and petals. Hairs are frequently of considerable importance in determining relationships within tribes, but in this group, where so few species produce hairs in any number on the floral parts and where these species are so different in other respects, not too much emphasis can be placed on them.

When these features, as exemplified in the species of the Andromedeae, are considered, certain patterns of relationships, which are summarized in table 2,

TABLE 2



can be seen. Thus *Epigaea*, *Enkianthus*, *Oxydendrum*, and probably *Chamaedaphne* occupy fairly isolated positions while the rest are placed in two large groups: one containing *Leucothoe*, *Pieris*, *Ampelothamnus*, *Lyonia*, *Xolisma*, and

Neopieris, the other *Cassiope*, *Harrimanella*, and *Andromeda*. Within these groups further subdivision can be made; thus *Andromeda* is to be separated from *Cassiope* and *Harrimanella*, while in the latter group *Harrimanella* is rather distinct, and *C. hypnoides* occupies a position somewhat intermediate between it and the other three species of *Cassiope*. In the other big group three subdivisions can be made: (*a*) the four species of *Leucothoe*, (*b*) *Pieris* and *Ampelothamnus*, and (*c*) *Lyonia*, *Xolisma*, and *Neopieris*. *Chamaedaphne* might possibly be placed here as a fourth subdivision. Further subdivisions can be made in the first and third of these.

Epigaea is distinct as a result of a number of different characters: the fact that it is functionally dioecious, the corolla funnel-form, the corolla and ovary covered with hairs, the long free limbs of the corolla, the double placenta in each carpel and the extent of parietal placentation in the top of the ovary, the septal location of ventral carpel bundles, the vascular supply to the nectariferous tissue which does not form a distinct region but is merely part of the ovary wall, the greatly expanded and lobed stigma, the relative lack of distinct staminal appendages, the large number of ovules, and the size, persistence, and vascular supply of the bracts on the pedicel. As many of these characters are found only in *Epigaea* and not in any other genera, this genus occupies a decidedly isolated position in this group. This has been suggested in classifications which include it in a separate tribe, the Epigaeae (**7**).

Enkianthus has a few characters in common with *Epigaea*, such as the septal position of ventral carpel bundles, apparent lack of a distinct nectariferous enlargement, and type of sepal supply, but differs from it and the others in the actual position in the septum of the septal carpel bundles, the reversal of the reflexion of traces—the median sepal trace tapering upwards and the dorsal carpel strongly reflexed—the origin at different levels of the median and lateral sepal traces, the relatively low number of ovules, and the nondivergence of vascular traces occurring only in the sepalad stamen-septal carpel supply.

Oxydendrum has the only flower which shows a tapered receptacle with traces to all floral organs completely distinct and the petalad stamen trace not diverging until above the closure of the petal gap. The ovary is deeply depressed, and the placenta, which is attached near the bottom, extends upward in each locule. There is little modification of the ovary base in connection with the nectariferous tissue. The origin of its sepal supply is similar to that found in the other isolated genera—*Epigaea*, *Enkianthus*, and *Chamaedaphne*, and a few of the *Pieris* and *Lyonia* groups—but otherwise *Oxydendrum* does not have much in common with any of these. The hairs, which occur on young buds, appear to be different from those in *Epigaea*. The upper portions of the connective show a disinte grating region extending into the pair of short awns which occur on each anther.

Chamaedaphne is not so isolated as the three preceding genera. It has the three-trace sepal of the group including *Leucothoe* and *Pieris*, but the heavy vascular supply which is similar to the condition in *Pieris* is not accompanied by the fiber strands characteristic of the latter genus. There is no reflexion of vascular pathways, though there is some nondivergence of traces. It has scales on the two large persistent bracts and the calyx, and the nectary has long free lobes between the stamens. The only suggestion of staminal appendages is the presence of a

pair of short awns on each anther. It also shows an abortion of ovules, or of microsporogenous tissue in many flowers.

The isolated species and the *Leucothoe-Lyonia-Pieris* group all have three traces entering the base of the sepal. The latter group, with *Chamaedaphne*, have essentially completely sympetalous, urceolate corollas and floral parts which are almost flat or mildly reflexed at the base. The species of *Leucothoe* have one character in common which separates them from all other species studied—the position of the placentae against the outer carpel walls with the ovules borne on the inside surfaces. The ovules are fairly large and relatively few in number. There are no spurs on the stamens of any of them, and they all have disintegrating areas developing in the connective lobes. They also have a pair of bracts just below the flower.

Pieris and *Ampelothamnus* have in common the fiber strands in the sepals which are found in no other species, and stamens with large spurs which show no disintegration tissue and have connective lobes with granular pouches. The filaments are greatly expanded at the base and vertically S-shaped in the flower. The nectary has upward lobes from a narrow base on the ovary or on the receptacle.

The two species of *Lyonia*, *Xolisma*, and *Neopieris* have filaments which are horizontally S-shaped in the flower, spurs which show disintegrating tissue, and a very short pair of awns on each anther. They all show a considerable nondivergence of vascular strands to different organs; and the nectary is only an enlargement of the ovary wall (except in *Neopieris* where little nectariferous tissue occurs).

Leucothoe can be separated into two groups of two species each. *L. axillaris* and *L. catesbaei* have a single trace to each sepal which immediately divides to form three, while *L. racemosa* and *L. recurva* have three traces, all arising independently. The latter two species have four long, awnlike extensions per anther and filaments which are expanded at the base, while the former two do not. The nectary characters vary in all species.

Ampelothamnus differs so little in most characters from the two species of *Pieris* that it might well be included in the same genus.

Xolisma is the only one of the latter group which has a heavy growth of hairs; *Lyonia mariana* is peculiar in its massive placentae, each with a very large number of small ovules. *L. ligustrina* and *Xolisma* have a short, strongly urceolate corolla, while in *L. mariana* and *Neopieris* it is almost bell-shaped. The spurs in *Neopieris* are long, while those in *Lyonia* and *Xolisma* are very short. *L. mariana* is the only species studied which showed a reduplicate aestivation (except for very young buds of *Pieris*).

Cassiope, *Harrimanella*, and *Andromeda* are characterized by a single trace to the sepals (except in *C. hypnoides*), strong reflexion of the floral organs and associated vascular traces, considerable nondivergence of traces to different organs, and a fairly massive nectariferous area on the base of the ovary (except in *Harrimanella*, where it appears to be receptacular) with free tissue up, down, or both.

Cassiope and *Harrimanella* differ from *Andromeda* in the position of anther-filament attachment which is at the top in the former and at a midpoint in the latter; also the anther position approaches the horizontal in the former (in the bud it is actually almost vertical in the noninverted position) and is vertical (in the inverted position) in the latter. Stamens

in *Cassiope* and *Harrimanella* are characterized by spurs and in *Andromeda* by pairs of well-developed awns. The ovule number and size are different—few and large in *Andromeda*, many and small in *Cassiope* and *Harrimanella;* stigmas are expanded in the former and narrow in the latter. The difference in corolla shape and in vegetative characters also adds to the separation of these two.

Harrimanella and *C. hypnoides* differ from *Cassiope lycopodioides*, *C. mertensiana*, and *C. tetragona* in the possession of spurs which do not show disintegration tissue, the relatively long free lobes of the corolla, and the shape of the placenta with only a strong downward lobe. *Harrimanella* differs from all species of *Cassiope* in its nectary characteristics and the almost complete independence in origin of traces to different organs.

There are only minor vascular differences between *Andromeda glaucophylla* and *A. polifolia*, and study of samples from a variety of areas might well show an overlap in these characters.

The grouping of the genera given above is tentative. Work on other aspects of floral morphology, particularly the details of stamen development and of the ovules, is contemplated as soon as the necessary collections can be completed. Data from such a study correlated with the above, with vegetative anatomy, and with the usual taxonomic characters, should help to clarify the relationships and nomenclature in the Andromedeae.

Summary

1. Twenty-two species of the Andromedeae are described with respect to the organography and vascular anatomy of the flower: *Ampelothamnus phyllireifolius*, *Andromeda glaucophylla*, *A. polifolia*, *Cassiope hypnoides*, *C. lycopodioides*, *C. mertensiana*, *C. tetragona*, *Chamaedaphne calyculata*, *Enkianthus cernuus*, *Epigaea repens*, *Harrimanella stelleriana*, *Leucothoe axillaris*, *L. catesbaei*, *L. racemosa*, *L. recurva*, *Lyonia ligustrina*, *L. mariana*, *Neopieris nitida*, *Oxydendrum arboreum*, *Pieris floribunda*, *P. japonica*, and *Xolisma fruticosa*.

2. The Andromedeae are pentacyclic (the outer whorl of stamens opposite the petals), pentamerous, sympetalous, hypogynous, and perfect (except for *Epigaea*, which is functionally dioecious). The stamens have introrse anthers, and most have appendages: spurs and/or awns of varying character. The corolla varies from funnel-form through bell-shape to extremely urceolate. The calyx and corolla in many are strongly reflexed at the base of the flower. The placentation is in general axile but may become parietal at the top of the depressed ovary.

3. The vascular system in the pedicel forms a complete cylinder. It usually broadens in the receptacle and is broken by gaps above the traces to the various organs. The following whorls of traces arise in acropetal order: sepals, petals, petalad stamens, sepalad stamens, dorsal carpels in the plane of the petals, septal carpels in the plane of the sepals, and the ventral carpels or placentals. There are different degrees of nondivergence of various of these whorls; most frequently the petal and petalad stamen or sepalad stamen and septal carpel traces are common in origin.

4. Traces to a sepal and septal carpel region may be from one to three; they are single to all other organs. The sepal supply is of four types: (*a*) three traces arise to supply each sepal, so that a total of fifteen gaps occur in the vascular cylinder; (*b*) lateral sepal traces of adjacent sepals have a common origin, so that each sepal receives three traces and ten gaps are formed; (*c*) a single trace

for each sepal arises and divides immediately into three—each sepal receives three traces, but only five gaps occur; and (*d*) a single trace to each sepal.

5. The dorsal carpel bundles form the stylar vascular supply; the septal traces fade out without reaching the style; and the ventral carpels supply the placentae and ovules. The latter bundles normally occur in the plane of the locules but in two species are found in the plane of the septa.

6. A tentative grouping of the species studied, based only on the facts of organography and vascular anatomy described, is given in table 2. The initial separation is on the basis of sepal supply; additional characters are used to subdivide the groups.

The author wishes to express her gratitude to all who gave of their time and energy in collecting the materials which helped to make this paper possible.

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THE TETRASPORIC EMBRYO SACS OF PLUMBAGO COCCINEA, P. SCANDENS, AND CERATOSTIGMA WILLMOTTIANUM

J. W. BOYES AND E. BATTAGLIA[1]

The occurrence of a tetrasporic embryo sac of the *Plumbago* type (**4**) in *Plumbago coccinea* Salisb., *P. scandens* L., and *Ceratostigma willmottianum* Stapf. was briefly reported by one of the authors about 10 years ago (**1**). More recently the present status of our knowledge of embryo-sac development in the Plumbaginaceae was summarized by the authors (**2**). This paper is a more extensive account of embryological observations in *Plumbago coccinea*, *P. scandens*, and *Ceratostigma willmottianum*. For material and methods see BOYES (**1**).

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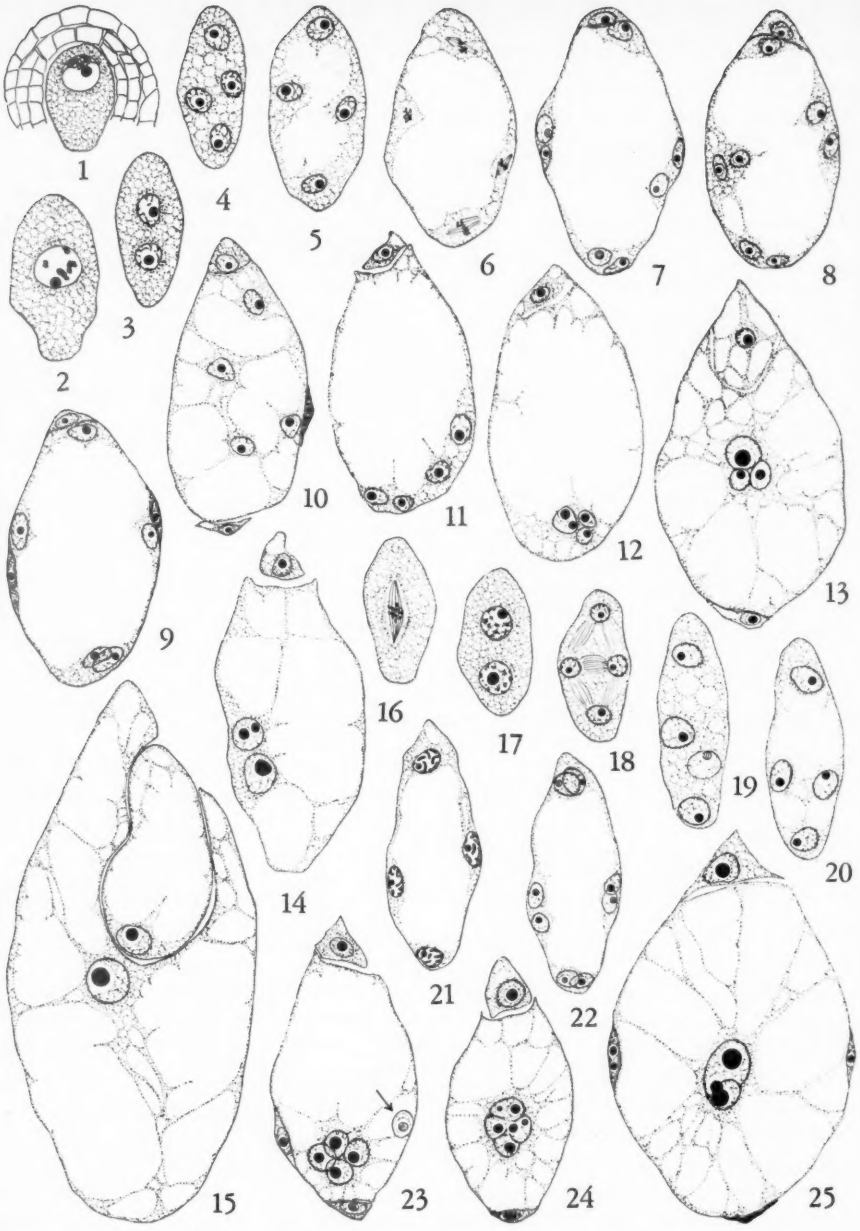
Observations

PLUMBAGO COCCINEA

General observations concerning the structure and development of the ovule have confirmed previous conclusions of DAHLGREN (**3**) and HAUPT (**4**). In the early meiotic prophase the megaspore mother cell is covered by a layer of



FIGS. 1–25.—Figs. 1–15.—*Plumbago coccinea*, stages in development of embryo sac; × 455. Fig. 1, megaspore mother cell; fig. 2, same at diakinesis; fig. 3, two-nucleate stage; fig. 4, tetranucleate stage at beginning of vacuolation; fig. 5, same with more vacuolation and four sporic nuclei showing polarization follow-

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parietal cells (fig. 1). It enlarges considerably during the heterotypic prophase, as is evident in comparison of figures 1 and 2 (the latter at diakinesis). Meiosis I results in two free nuclei which pass into a brief interkinesis stage (fig. 3). Meiosis II, which follows immediately, ends with the formation of four megaspore nuclei arranged so that each of them occupies one of the four terminal points of the longitudinal and transverse axes of the embryo-sac mother cell (fig. 4). A period of growth of the embryo sac follows, during which many vacuoles appear in the cytoplasm. By confluence the vacuoles give rise finally to a large vacuole that occupies most of the embryo sac (fig. 5). The four megaspore nuclei, retaining their initial arrangement, undergo a division (fig. 6) resulting in the formation of an eight-nucleate embryo sac (fig. 7). The daughter nuclei remain together in pairs, and promptly the outer nucleus of each pair is cut off by a wall to form a uninucleate cell. This wall for the micropylar pair seems to be established before those of the remaining nuclei (figs. 7, 8). The same occurs in *P. capensis* (**4**: fig. 17).

At the end of this wall-formation process four small uninucleate cells are present, lying at the extremities of the two axes of the embryo sac (fig. 9). The uninucleate cell at the micropylar end becomes the egg; the other three constitute two "lateral cells" and one antipodal. Most of the embryo sac is occupied by a large vacuolate cell containing four polar nuclei. The antipodal and the lateral cells quickly degenerate at their original location. In the case shown in figure 10 one lateral cell has precociously disappeared.

Subsequent development is characterized by a strong growth of the embryo sac. During such growth the four polar nuclei of the central cell gradually approach one another (figs. 11, 12) and fuse. Usually such fusion occurs initially between two of the nuclei (fig. 12) and later for the remaining two (fig. 14). More infrequently there is simultaneous union of two polar nuclei with a precedent fusion nucleus (of two polars as above) represented in figure 13. (The case shown in fig. 13 is also noteworthy for the exceptional persistence of the antipodal cell.) Thus a tetraploid "secondary nucleus" is formed (fig. 15). The typical last stage of the embryo sac is thus binucleate, i.e., provided only with an egg with a haploid nucleus and a central (proendospermatic) cell with a tetraploid secondary nucleus.

The development type of *P. coccinea* is very similar to that first described by HAUPT (**4**) in *P. capensis* (*Plumbago* type).

PLUMBAGO SCANDENS

As in the preceding species the megaspore mother cell through two meiotic divisions gives rise to a tetrasporic embryo sac (figs. 16–20). Following the division

ing the scheme $1 + \frac{1}{1} + 1$; fig. 6, metaphase of first somatogenic division (last division of development); fig. 7, eight-nucleate stage; fig. 8, eight-nucleate stage in which egg and one lateral cell have been cut off by walls; fig. 9, eight-nucleate stage showing wall formation nearly complete; fig. 10, same in which a lateral cell has already disappeared; figs. 11–14, progressive migration and fusion of four polar nuclei (see text); fig. 15, typical aspect of mature embryo sac. Figs. 16–25.—*Plumbago scandens*, stages in development of embryo sac; × 455. Fig. 16, metaphase I; fig. 17, two-nucleate stage; fig. 18, late telophase II, megaspore nuclei are connected by "secondary fibers"; figs. 19, 20, four-nucleate stages progressively developed; fig. 21, first somatogenic division (prophase); fig. 22, eight-nucleate stage; fig. 23, same showing four polar nuclei lying near one another (lateral cell, which simulates a supernumerary polar nucleus, is indicated by arrow); fig. 24, nearly mature embryo sac in which two lateral cells have disappeared (unusual); fig. 25, typical, almost mature embryo sac with antipodal and lateral cells still persisting.

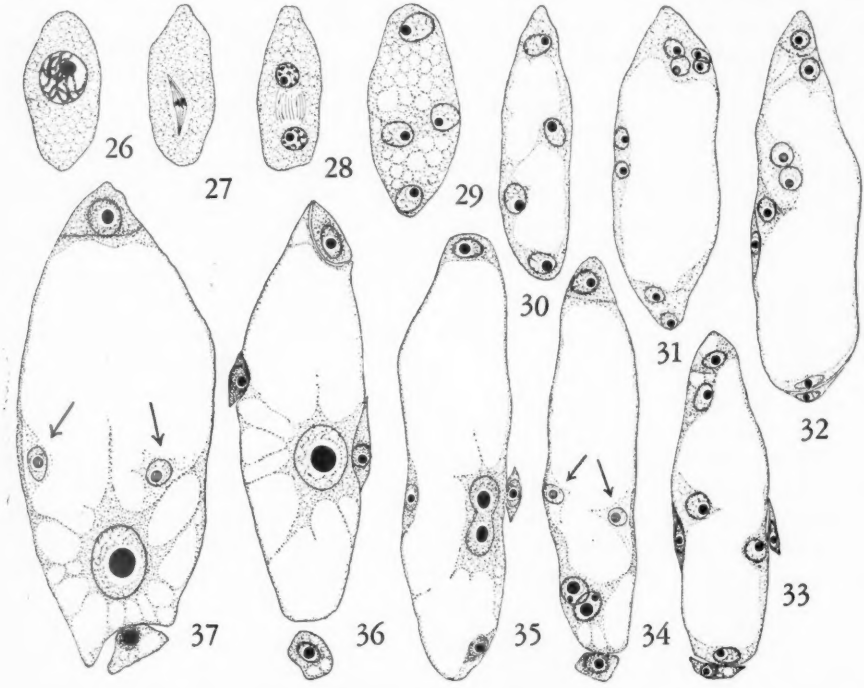
of each of the four megaspore nuclei (fig. 21), which exhibit an arrangement at the points of a cross, an eight-nucleate embryo sac is formed (fig. 22).

After this stage walls are formed between the nuclei of each pair, and thus an egg, two lateral cells, an antipodal cell, and the proendospermatic cell with four polar nuclei become differentiated. Soon the polar nuclei migrate toward the center of the proendospermatic cell and fuse to produce a tetraploid secondary nucleus (figs. 23, 24, 25).

In contrast to what was described above in *P. coccinea* the lateral and the antipodal cells of *P. scandens* usually persist in the mature embryo sac. Sometimes, however, one or two lateral cells may disappear precociously (fig. 24). More rarely one finds degeneration of the antipodal cell (fig. 25). Usually the wall which separates the two lateral cells from the central cell seems to be very thin, and thus the nuclei of these cells simulate supernumerary polar nuclei (fig. 23, indicated by arrow).

CERATOSTIGMA WILLMOTTIANUM

In this species the meiotic divisions of the spore mother cell (figs. 26–29), vacu-



FIGS. 26–37.—*Ceratostigma willmottianum*, stages in development of embryo sac; × 455. Fig. 26, megaspore mother cell; fig. 27, metaphase I; fig. 28, late telophase I; fig. 29, tetranucleate stage; fig. 30, tetranucleate vacuolate stage, the sporic nuclei polarized following the scheme $1 + \frac{1}{1} + 1$; figs. 31, 32, eight-nucleate stages with fig. 31 showing four nuclei abnormally appearing at micropylar end of embryo sac; fig. 33, eight-nucleate stage after wall formation, showing two distinct lateral cells, one antipodal cell, and an egg differentiated; figs. 34, 35, progressive stages in secondary nucleus formation (arrows indicate lateral cells); figs. 36, 37, mature embryo sacs of usual appearance, i.e., with large antipodal cell and two clearly differentiated lateral cells.

olations, polarization (figs. 29, 30), and the first somatogenic division (last division of development) proceed as in the previous two species. In the eight-nucleate stage thus formed the nuclei lie in pairs at the four points of a cross. Exceptionally, two pairs may be located in the micropylar part of the embryo sac (fig. 31) or at one side (fig. 32). Wall formation follows, cutting off the egg, two lateral cells, and one antipodal cell (fig. 33).

C. willmottianum differs from the two *Plumbago* species of this study in that the lateral cells and the antipodal are larger and are clearly separate as cellular entities (figs. 33–36). The antipodal cell is very conspicuous (figs. 36, 37). Usually these lateral and antipodal cells persist until after formation of the secondary nucleus (figs. 34, 35, etc.). As mentioned for the embryo sac of *P. scandens*, the walls of the lateral cells are sometimes not visible in direct view, and then their nuclei simulate supernumerary polar nuclei (indicated by arrows in figs. 34, 37).

The mature embryo sac is large and typically possesses only a large egg and a uninucleate proendospermatic cell, the antipodal and lateral cells having disappeared. An exceptional late stage was found with abnormal occurrence of a supernumerary egg. This anomaly apparently originated as the consequence of an abnormality like that shown in figure 31.

Summary

1. The embryo sacs of *Plumbago coccinea* Salisb., *P. scandens* L., and *Ceratostigma willmottianum* Stapf. develop following the *Plumbago* type, as first correctly described by HAUPT (4) in *Plumbago capensis*.

2. Among the three species investigated, certain differences in the behavior of the lateral and antipodal cells have been observed.

This work was started while the senior author held a scholarship from the Wisconsin Alumni Research Foundation. It is a pleasure to acknowledge this financial assistance and the kindly encouragement of Dr. C. E. ALLEN.

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DEVELOPMENT OF THE TETRASPORIC EMBRYO SAC OF CHRYSANTHEMUM VISCOSUM

EMILIO BATTAGLIA

Introduction

Since the extensive embryological reinvestigations of FAGERLIND (3) and MAHESHWARI and HAQUE (6) on *Chrysanthemum parthenium* (L.) Bernh. (*Pyrethrum parthenifolium* var. *aureum*), interest in this genus has grown. PALM (8) first discovered the presence of a tetrasporic embryo sac in *Chrysanthemum parthenium* and gave a detailed account of its development. CHIARUGI (2), on the basis of PALM's work and the observation of similar development in other plants, named this the "tipo *Pyrethrum* 16-nucleato." In this type the meiotic divisions form four megaspore nuclei which are not separated from one another by walls. Then the embryo sac gradually increases in size and elongates. During this growth vacuoles soon arise between the linearly arranged nuclei (polarization 1 + 1 + 1 + 1). In these respective positions the nuclei divide simultaneously twice, resulting in a sixteen-nucleate stage. The four micropylar nuclei (produced by divisions of the micropylar nucleus of the tetranucleate stage) give rise to synergids, egg cell, and upper polar nucleus. The remaining nuclei, occupying the elongated basal part of the embryo sac, form the lower polar nucleus and eleven antipodal cells. Occasionally the number of antipodal cells may be less than eleven because certain of them may contain two nuclei.

The plants of *C. parthenium* collected at the Brooklyn Botanic Garden and studied by MAHESHWARI and HAQUE (6) showed an embryological development similar to that described above. FAGERLIND (3), however, found embryo-sac development to differ in plants from two sources, though tetrasporic in both cases. In plants from the Hortus Bergianus of Stockholm the four megaspore nuclei divided first to produce eight; of these eight nuclei, the two basal ones which were the smallest failed to undergo the next and last division, resulting in an embryo sac with fourteen nuclei. The fully organized embryo sac contained two synergids, one egg, and two polar nuclei, and in the chalazal part, uninucleate upper antipodal cells and multinucleate (later polyploid in one case) lower ones. Occasionally the number of nuclei in the final stage was less than fourteen because of precocious degeneration of the undivided nuclei during the last division. Typical sixteen-nucleate stages were also produced. In the other plants studied by FAGERLIND, material collected by Dr. HOLMGREN, the basal nucleus of the tetranucleate stage showed early degeneration; the remaining three nuclei divided twice, thus forming a final twelve-nucleate stage.

Such interesting variability in embryo-sac development clearly indicates the desirability of extending the embryological studies in this genus.

Material and methods

Buds of *Chrysanthemum viscosum* L. were collected from living plants in the Botanical Garden of Pisa University

(Italy). Navashin's fixative, with a 10-second pretreatment in Carnoy's solution, was used for fixation. All material was dehydrated in alcohol, cleared in chloroform, imbedded in paraffin, sectioned at 25 μ, and stained in Heidenhain's iron-alum haematoxylin.

Observations

The diploid chromosome number of *C. viscosum* was determined from the root-tip cells as $2n = 18$ (fig. 1). The haploid complement ($n = 9$) was confirmed during megasporogenesis (figs. 3, 11). Microsporogenesis (simultaneous type) and development of the male gametophyte are quite regular.

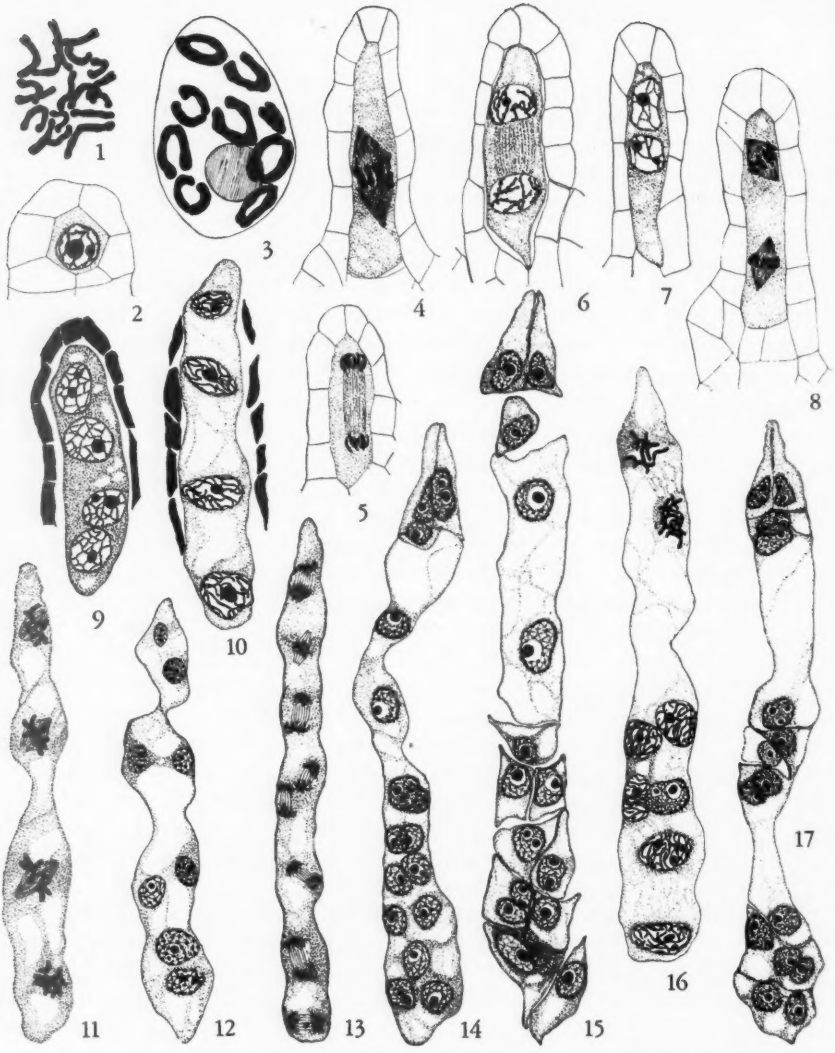
Development usually follows the *Pyrethrum parthenifolium* type. As in other Compositae the ovule is unitegmic and the archesporium one-celled. The hypodermal archesporial cell functions directly as the megaspore mother cell (fig. 2). As a result of the first meiotic division (figs. 3–6) two nuclei of equal size are formed (fig. 7). Both undergo the second meiotic division (fig. 8), and the resulting four nuclei take up a linear arrangement (fig. 9). Vacuolation takes place at this stage, and the four nuclei become separated from one another by vacuoles without losing their linear arrangement (polarization 1 + 1 + 1 + 1) (fig. 10). The four nuclei are usually of almost equal size, but sometimes the micropylar one is smaller (fig. 10). The next division, simultaneous for all megaspore nuclei (fig. 11), gives rise to eight nuclei that lie in pairs, each pair being separated from the next by a vacuole (fig. 12).

The last nuclear division is characterized by the absence of strict mitotic simultaneity. Often the start of mitosis is precocious in the micropylar pair (fig. 16), but not in all cases (fig. 13). The ensuing resting stage shows sixteen nuclei (fig. 14). During wall formation the embryo sac organizes two synergids, an egg cell, a proendospermatic cell with the two polar nuclei, and a variable number of antipodals. Sometimes wall formation occurs precociously in the micropylar portion of the embryo sac (fig. 14). The theoretical number of eleven uninucleate antipodal cells was observed. Frequently only nine antipodal cells occur, two of them binucleate (fig. 15). In one case the embryo sac had only five antipodal cells, two uninucleate and the remaining with two, three, and four nuclei, respectively. In another case six uninucleate antipodal cells plus a two- and a three-nucleate cell were present. Different combinations have been observed in the organization of antipodal cells, but in all cases the total number was between five and nine, usually with two or three antipodals in the multinucleate condition.

Thus, the usual development of the embryo sac follows the pattern described first by PALM (**8**) in *Pyrethrum parthenifolium* var. *aureum*. The "*Pyrethrum parthenifolium* type" based on PALM's work, was established by CHIARUGI (**2**). Other authors, for example, MAHESHWARI (**4, 5**), prefer the term "*Drusa* type."

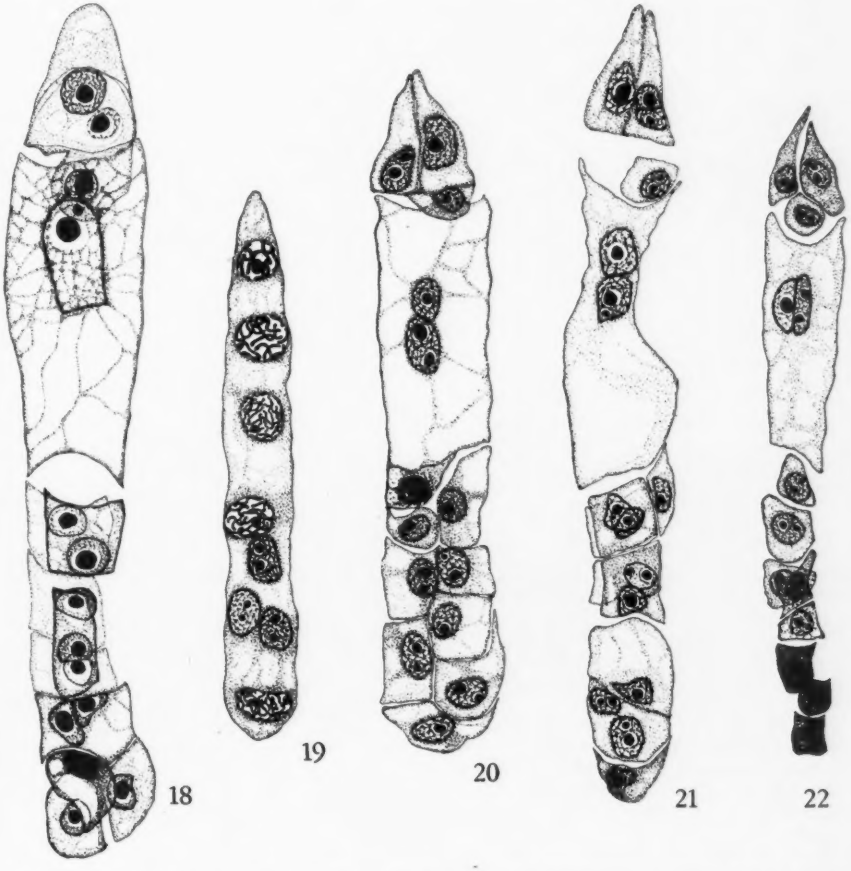
Development occasionally follows the *Pyrethrum parthenifolium* subtype 15-nucleate. Sometimes a chalazal nucleus in the eight-nucleate stage fails to undergo the last division (fig. 16). There follows a mature gametophyte with fifteen nuclei (figs. 17, 18). In figure 17 only five antipodals are present: two one-nucleate, two three-nucleate, and one two-nucleate. In the embryo sac shown in figure 18, however, there are nine antipodals, only one of them in the binucleate condition.

Development also occasionally follows the *Pyrethrum parthenifolium* subtype 14-nucleate or subtype 13-nucleate. That



FIGS. 1–17.—*Chrysanthemum viscosum*. Fig. 1, somatic chromosomes from root tip cell, $2n = 18$, camera lucida drawing; × 1400. Figs. 2–17, stages in development of embryo sac, camera lucida drawings; fig. 3 is × 1500, the rest × 550. Fig. 2, nucellus with archesporial cell; fig. 3, megaspore mother cell in diakinesis (9 bivalents); fig. 4, same in metaphase I; figs. 5, 6, early and late telophase I; fig. 7, interkinesis; fig. 8, metaphase II; fig. 9, four megaspore nuclei; fig. 10, same in prophase of first somatogenic division; fig. 11, same in metaphase; fig. 12, eight-nucleate stage with nuclei in pairs; fig. 13, last division (mostly anaphases) in embryo-sac development; fig. 14, sixteen-nucleate, wall formation still absent in chalazal part of embryo sac; fig. 15, sixteen-nucleate stage showing organization of antipodals (see text); fig. 16, eight-nucleate stage showing nonsimultaneous beginning of last division with one chalazal nucleus quiescent; fig. 17, fifteen-nucleate stage (five antipodal cells).

two or three nuclei of the eight-nucleate stage may fail to take part in the last division of the gametophyte is clearly demonstrated in figure 19 where three chalazal nuclei are in resting stage while the remaining five show a more or less advanced prophase. One of the three may perhaps show the beginning of prophase. If only two chalazal nuclei fail to undergo this last division, the mature gametophyte would attain a fourteen-nucleate condition. Such embryo sacs have been observed (figs. 20, 21). When three chalazal nuclei fail to undergo the last division, a final thirteen-nucleate stage arises (fig. 22). There may exist a relationship between the absence of a division at the eight-nucleate stage and the precocious degeneration of antipodals in mature embryo sacs. Thus in figure 18 there is only



FIGS. 18–22.—*Chrysanthemum viscosum*, stages in embryo-sac development, camera lucida drawings; × 610. Fig. 18, fifteen-nucleate stage with nine antipodals; two polar nuclei have fused; fig. 19, eight-nucleate stage showing asynchronous beginning of last division; fig. 20, fourteen-nucleate stage (nine antipodals); fig. 21, same but with eight antipodal cells; fig. 22, thirteen-nucleate stage with eight antipodals.

one precociously degenerated antipodal, while in figure 22 there are three such cells.

Discussion

The development as above outlined for the sixteen-nucleate cases constitutes an embryo-sac type at present called *Pyrethrum* type by CHIARUGI (2) and *Drusa* type by MAHESHWARI (4, 5). The author prefers CHIARUGI's term for evident priority. In addition, the peculiarities of development described in *Pyrethrum parthenifolium* (*Chrysanthemum parthenium*) by PALM (8) and FAGERLIND (3) denote a coexistence of the general type of development with subtypes originating in most cases by the absence of the last division in one or more nuclei of the eight-nucleate stage. Such variation clearly occurs also in *Chrysanthemum viscosum*. Thus it seems natural and legitimate to use, for the sixteen-nucleate type, the term "*Pyrethrum parthenifolium* 16-nucleate,"[1] and for the variations brought about by the absence of the last division in one or more nuclei in the eight-nucleate stage the term "subtype" followed by the total number of nuclei; for example, "*Pyrethrum parthenifolium* subtype 15-nucleate," "14-nucleate," etc. These problems in terminology will be treated more extensively in future work.

[1] The addition of the name of the species is necessary because there exists also the *Pyrethrum cinerariaefolium* type (1, 7).

Summary

1. The diploid chromosome number of *Chrysanthemum viscosum* L. is eighteen.

2. The embryo sac is tetrasporic and usually develops a final sixteen-nucleate stage following the "*Pyrethrum parthenifolium* 16-nucleate" type.

3. Sometimes one or more nuclei of the eight-nucleate stage do not take part in the last division, and thus final stages arise with fifteen, fourteen, or thirteen nuclei (*Pyrethrum parthenifolium* subtypes 15-, 14-, 13-nucleate).

The author wishes to express his deep indebtedness for help in the English translation to Professor J. W. BOYES and Dr. E. R. BOOTHROYD.

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THE EMBRYO SAC OF ERYTHRONIUM AMERICANUM[1]

ASHRAFUL HAQUE

Introduction

Among the various kinds of tetrasporic embryo sacs the *Adoxa* type was formerly considered to be the most common. In this there is only one division of the four megaspore nuclei. The mature embryo sac is eight-nucleate: a three-celled egg apparatus, three antipodal cells, and two polar nuclei. The earliest reports of this type of embryo sac were made almost simultaneously by JÖNSSON (**13**) in *Adoxa* and TREUB and MELLINK (**21**) in *Lilium* and *Tulipa*. The researches of BAMBACIONI (**1, 2**) have shown, however, that development in *Lilium* and *Fritillaria* is quite different. She found that soon after meiosis the four megaspore nuclei take up a 1+3 arrangement, one nucleus at the micropylar end and three at the chalazal end of the cell. The micropylar nucleus divides in a normal fashion, but the spindles of the three chalazal nuclei fuse. As a result there is a second four-nucleate stage with two haploid nuclei at the micropylar and two triploid nuclei at the chalazal end of the embryo sac. A single subsequent division results in a mature sac in which the three cells of the egg apparatus are haploid, the three antipodal cells triploid, and the polar fusion nucleus tetraploid. This is the *Fritillaria* type of embryo sac.

Recent studies have shown that many of the embryo sacs formerly believed to be of the *Adoxa* type are really of the *Fritillaria* type; consequently, the *Fritillaria* list has gone on increasing, while the *Adoxa* list has diminished until now it comprises only four genera: *Adoxa*, *Sambucus*, *Erythronium*, and *Tulipa* (**15, 16, 17, 18**).

In the genus *Erythronium* both the *Fritillaria* and the *Adoxa* types of development have been reported. SCHAFFNER (**20**) reported the "Lilium type" (*Adoxa* type in current terminology) in *E. albidum* and *E. americanum*. HRUBÝ's (**10, 11**) work on *E. dens-canis*, however, indicated that the development here is of either the *Drusa* or the *Fritillaria* type, and this led MAHESHWARI (**14**) and FAGERLIND (**7, 8**) to doubt SCHAFFNER's account. COOPER (**5**), on the other hand, confirmed SCHAFFNER regarding the occurrence of the *Adoxa* type in *E. albidum*. OIKAWA (**19**), working on the only Eurasian species, *E. japonicum*, demonstrated that the development in this species is of the *Fritillaria* type. CAVE (**3**) also found the *Fritillaria* type of embryo sac in *E. helenae* and *E. tuolumnense*.

In a recent review of the *Adoxa* type of embryo sac MAHESHWARI (**15**) summarized the observations of COOPER (**5**) on *E. albidum*, and in the light of these he remarked that SCHAFFNER's (**20**) report of an occurrence of the *Adoxa* type in *E. americanum* may perhaps be correct, "but this needs confirmation." The present study was undertaken at his suggestion.

Material and methods

Ovaries at different stages of development were sent in formalin-acetic–alcohol by Dr. H. W. BANNAN of the Univer-

[1] A preliminary account of this investigation appeared in the *Proceedings of Indian Science Congress, 36th Session* (**9**).

sity of Toronto to Professor P. MAHESHWARI. The material was imbedded in the usual way, and transverse sections cut at 14–18 μ were stained with iron-alum haematoxylin.

Observations

NORMAL DEVELOPMENT

The ovary is trilocular, each locule containing two rows of anatropous, bitegmic ovules. The nucellar epidermis remains intact until the eight-nucleate stage of the embryo sac. The outer integument is made up of four layers of cells and the inner of three layers. One of the subepidermal cells of the nucellus becomes differentiated as the primary archesporial cell. This enlarges, becoming two to three times as long as broad, and functions directly as the megaspore mother cell. Its cytoplasm is finely vacuolate throughout.[2] The nucleus occupies an approximately central position (fig. 1). The heterotypic spindle usually lies parallel to the long axis of the cell, although sometimes it may be oriented somewhat obliquely or even transversely (fig. 2). Twenty-four pairs of chromosomes have been counted both at metaphase (fig. 2) and at anaphase (fig. 3*A*, *B*, *C*). Spindle fibers between the two daughter nuclei sometimes persist even after the latter have passed into the interkinesis stage (fig. 4). Normally each of the two daughter nuclei is much smaller than the nucleus of the megaspore mother cell.

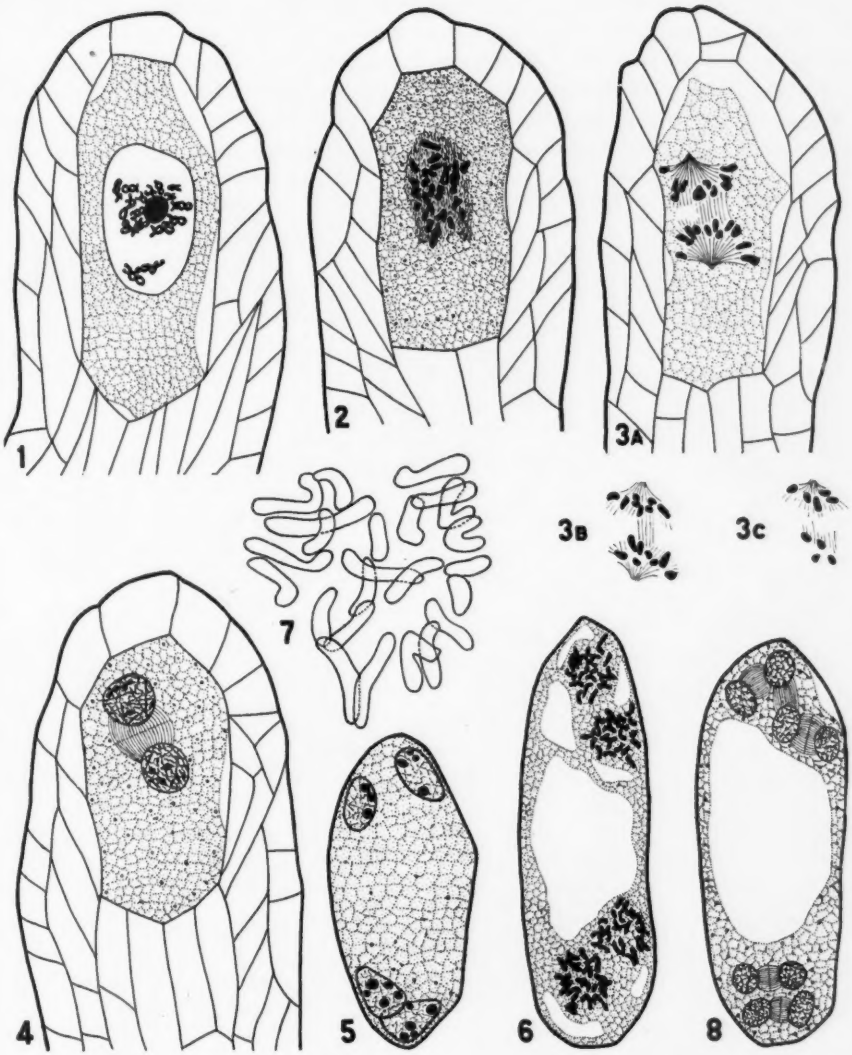
The spindles of the homeotypic division are usually oriented somewhat obliquely to the axis of the cell. The four nuclei resulting from this division (fig. 5) undergo a typical mitosis, and twenty-four chromosomes were counted on each metaphase plate (figs. 6, 7). COOPER (5) reported that in *E. albidum* the chalazal nucleus "enters upon a more or less abortive division" in which "masses or aggregations of chromatic material, rather than definite chromosomes, appear on the spindle." A similar abortive division resulting in two irregularly shaped nuclei occurs in *Lilium* (4). No such abnormality was found in the present case.

All eight nuclei formed after the last division are haploid. Spindle fibers between the daughter nuclei persist for a long time, and occasionally new fibers may arise between adjacent nuclei (fig. 8). COOPER (5) has reported the same condition in *E. albidum*.

Material at the writer's disposal did not show any embryo sac in which the eight nuclei had organized into an egg apparatus, antipodal cells, and polar nuclei. COOPER (5) stated that in *E. albidum* a definite organization of the embryo sac lasts for a short period only and that if fertilization does not take place during this time the membranes delimiting the cells disintegrate, leaving the nuclei free.

A very interesting occurrence was the presence of pollen grains inside a number of ovaries. The pollen grains showed a degenerating vegetative nucleus and a large spherical generative cell (figs. 9, 10). The presence of intra-ovarial pollen grains was first reported by JOHRI (12), who figured seven pollen grains in a carpel of *Butomopsis lanceolata*. In the present material sections of only part of an ovary mounted on a single slide showed more than one hundred and thirty pollen grains.

[2] Ordinarily the megaspore mother cell has a dense cytoplasm without any conspicuous vacuolation. There are only a few exceptions to this, for example, *Sambucus* and *Limnanthes* (8).

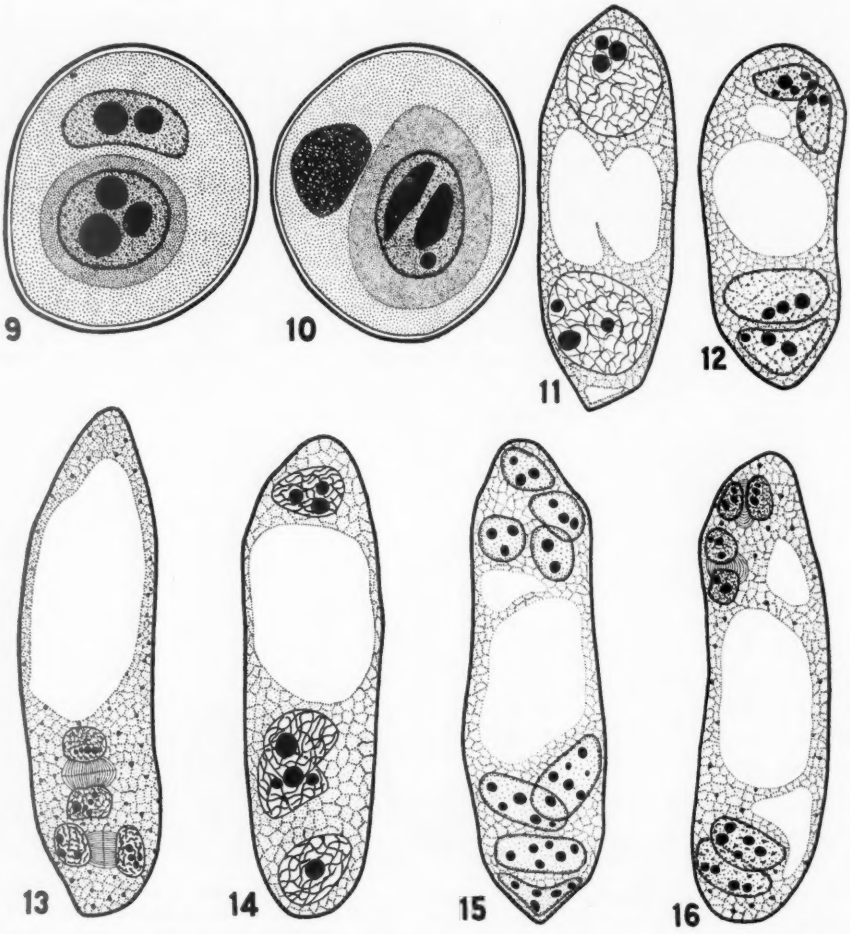


FIGS. 1–8.—*Erythronium americanum*. Figs. 1–6, 8, ×313. Fig. 7, ×1266. Fig. 1, megaspore mother cell with diplotene chromosomes. Fig. 2, megaspore mother cell with twenty-four bivalents in polar view of metaphase. Figs. 3*A*, *B*, *C*, megaspore mother cell at anaphase, adjacent sections. Fig. 4, two-nucleate sac. Fig. 5, four-nucleate sac. Fig. 6, third metaphase in embryo sac; all four chromosome plates are seen in polar view. Fig. 7, metaphase plate of third nuclear division in embryo sac showing twenty-four chromosomes. Fig. 8, eight-nucleate embryo sac.

ABNORMALITIES

Several two-nucleate sacs were seen in which the two nuclei were considerably larger than those of the normal two-nucleate stage. In the majority of such cases vacuolation was also more advanced than in normal two-nucleate embryo sacs (fig. 11).

One embryo sac showed four nuclei all aggregated toward the chalazal end (fig. 13). A careful search for nuclei in the micropylar end showed none. This



FIGS. 9–16.—*Erythronium americanum*. Figs. 9, 10, intra-ovarial pollen grains. ×790. Fig. 9, with large and spherical generative cell and smaller vegetative nucleus. Fig. 10, older stage; vegetative nucleus is degenerating. Figs. 11–16, abnormal embryo sacs. ×330. Fig. 11, sac with two abnormally large nuclei separated by vacuole. Fig. 12, four-nucleate sac with two chalazal nuclei considerably larger than two micropylar ones. Fig. 13, four-nucleate sac with all nuclei lying at chalazal end. Fig. 14, four-nucleate sac showing 1+3 arrangement of nuclei. Fig. 15, eight-nucleate sac with four chalazal nuclei considerably larger than four micropylar ones. Fig. 16, sac showing four small nuclei at micropylar end but only two large ones at chalazal end.

unusual position might be explained by assuming that a large vacuole appeared near the micropylar end of the sac and crowded all megaspore nuclei toward the basal end. Several four-nucleate embryo sacs were observed, some of which showed a 1+3 arrangement (fig. 14); others showed the two nuclei at the chalazal end larger than the two at the micropylar end (fig. 12).

At the eight-nucleate stage a few embryo sacs were observed in which the four chalazal nuclei were much larger than the four micropylar ones (fig. 15). In a few others there were only two large nuclei at the chalazal end with four smaller ones at the micropylar end (fig. 16).

Frequently the cytoplasm of the four-, as well as eight-, nucleate embryo sacs showed many globular bodies of varying sizes which stained very deeply and showed a strong resemblance to small nucleoli (figs. 2, 4, 5, 8, 13, 16). The nature of these bodies could not be determined; similar structures have been figured by HRUBÝ (**10**).

Discussion

Some of the abnormalities described above show that the development is not always of the *Adoxa* type. The 1+3 arrangement of megaspore nuclei observed in several embryo sacs (fig. 14) is suggestive of the *Fritillaria* type; this is supported by (*a*) the probable second four-nucleate stage shown in figure 12 and (*b*) the eight-nucleate sacs in which the four chalazal nuclei are comparatively larger than the four micropylar ones (fig. 15). Unfortunately, an actual fusion of the three chalazal spindles was not observed, nor was the writer able to find any dividing nuclei which showed the triploid number of chromosomes at the chalazal end and the haploid number at the micropylar end. There seems no way, however, to account for the reported deviations from the *Adoxa* type except on the assumption that in a certain percentage of ovules development follows the *Fritillaria* type. In light of this interpretation the embryo sacs with four small nuclei at the micropylar end and two large nuclei at the chalazal end (fig. 16) must be regarded as having originated by an omission of the last division at the chalazal end.

According to SCHAFFNER (**20**), the chromosome number for *E. americanum* and *E. albidum* is $n = 12$ and $2n = 24$, but COOPER (**5**) reported $n = 22$ and $2n = 44$ in *E. albidum*. As already stated, I have found $n = 24$ and $2n = 48$ in *E. americanum*. Since *E. albidum* and *E. americanum* are closely allied species, this difference in chromosome number is rather surprising. Records of chromosome numbers in other species of *Erythronium* (**3, 6, 19**) seem to indicate that 12 is the basic number for the genus. *E. americanum* with $n = 24$ must then be considered a tetraploid species, or it may have both diploid and tetraploid races, in which case it is possible that SCHAFFNER's material came from a diploid race and mine from a tetraploid one. It is probable that a similar condition occurs in *E. albidum*, but this point deserves further study.

The presence of pollen grains inside the ovary is an interesting and unusual feature. Unfortunately the styles and stigmas had already been removed in this material at the time of fixation. Further study is planned using whole flowers.

Summary

1. The ovary of *Erythronium americanum* is trilocular, each locule containing two rows of anatropous, bitegmic ovules.

2. One of the subepidermal cells of the nucellus enlarges and functions directly as the megaspore mother cell.

3. Normally only three divisions intervene between the megaspore-mother-cell stage and the eight-nucleate sac; thus the development is of the *Adoxa* type.

4. The chromosome number is $n = 24$ and $2n = 48$.

5. Some embryo sacs show an unusual organization which indicates that occasionally the development may be of the *Fritillaria* type.

6. Numerous intra-ovarial pollen grains were found. These, like other pollen grains, usually showed a degenerating vegetative nucleus and a large spherical generative cell with deeply staining cytoplasm and prominent nucleus.

I am greatly indebted to Professor P. MAHESHWARI of the University of Delhi for suggesting this problem and for his guidance and interest throughout the course of the investigation. Thanks are also due to Dr. H. W. BANNAN of the University of Toronto, who kindly collected the material.

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SOME PHYSIOLOGICAL EFFECTS OF INSECTICIDES ON CITRUS FRUITS AND LEAVES[1]

E. T. BARTHOLOMEW,[2] WILLIAM S. STEWART,[3] AND GLENN E. CARMAN[4]

Introduction

Insecticides may be effective and at the same time cause no visible injury to the plants on which they are used. The possibility that the plant may sustain invisible (internal) injury is, unfortunately, all too often overlooked. Some of the less apparent injurious effects of insecticides on plants are interference with (*a*) loss or retention of water, (*b*) exchange of gases, (*c*) manufacture of foods, and (*d*) transfer of foods from one part of the plant to another. Insecticides also may be responsible for the accumulation of toxic residues in plant tissues. Any one or more of these factors may temporarily or permanently retard the growth of the plant, may lower its food value, or may even prevent its use as food. All these possibilities should be considered when using any of the hundreds of single substances or compounds now available as potential insecticides.

Since 1935 the Divisions of Plant Physiology and Entomology of the University of California Citrus Experiment Station have co-operated in studying the effects of insecticides on citrus fruit and leaf tissues. Between 1935 and 1945 work was confined largely to study of the effects of HCN (hydrocyanic acid) and petroleum-oil sprays (**2, 3, 4, 5, 6, 13, 14**). Since then the studies have been concerned with the effects, not only of oil spray, but also of several new insecticides. Some of the results of these later studies are here reported. They include the effects on juice quality of fruit and on dry matter of leaves. The purpose of this paper is not to present a general discussion or summary of the voluminous data in this field but to report additional information pertaining to the physiological effects of insecticides on citrus fruits and leaves.

Material and methods

JUICE-QUALITY TESTS.—The general need for insect pest control in most of the experimental citrus orchards prevented comparison of juice of fruit from treated and nontreated plots. Juice-quality studies were therefore confined largely to the relative effects of different insecticides. The studies reported here relate almost entirely to the three substances that proved to be most effective as insecticides: 1,1,1-trichloro-2,2-bis-(*para*-chlorophenyl)-ethane (DDT), o,o-diethyl-o-*para*-nitrophenyl thiophosphate (parathion), and petroleum oil.

DDT sprays were prepared by dissolving 1-2 pounds of technical DDT in 2–3 gallons of solvent consisting of 95 parts of kerosene and 5 parts of either Velsicol AR-60[5] (chiefly di- and trimethyl naphthalenes) or tetralin (tetrahydronaphthalene) in each 100 gallons of water. Parathion (25% wettable powder) was used at 1½–4 (usually 2 or 3) pounds per 100

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[5] Velsicol Corporation, Chicago, Illinois.

gallons of water. A light-medium grade of petroleum oil was commonly used at a dosage of $1\frac{1}{2}$–$1\frac{3}{4}$ gallons per 100 gallons of water.

The DDT and oil tests were made between December, 1945, and February, 1950; the parathion and oil tests were made between November, 1947, and February, 1950. Two to 10 months elapsed between application of insecticide and testing of juice.

Fruits for the two main juice-quality tests (tables 1, 4) were taken from 163 plots of eight to twenty (usually twelve) trees each. These plots were located in four areas in the south-central part of California and in nineteen different areas in the southern part of the state. Each fruit sample consisted of 96–200 (usually 96) fruits. Total soluble solids were determined with an Abbé refractometer. Total acidity was determined in terms of citric acid by titrating with a standardized solution of sodium hydroxide, with phenolphthalein as indicator.

Most juice tests were made on navel oranges. Results of tests made on Valencias were so similar to those made on navels that the two are not always segregated in the tables.

Two less extensive tests were made to determine the effects on Valencia orange juice of repeated applications of DDT on the fruit at short intervals and to determine the relative effects of oil and HCN on the quality of grapefruit juice. In the former test, two samples were not sprayed and two were sprayed with American Cyanamid Company's compound no. 899 (di-2-ethylhexyl phthalate).

ASCORBIC-ACID TESTS.—Ascorbic-acid (vitamin C) tests were made by the potentiometric 2,6-dichlorophenolindophenol method described by RAMSEY and COLICHMAN (12). These tests were primarily on juice of fruits from trees that had been sprayed with DDT or petroleum oil. Tests were made 6 weeks to $5\frac{1}{2}$ months (usually 4–5 months) after the insecticide had been applied.

LEAF DRY-MATTER TESTS.—Mature navel and Valencia orange leaves for these tests were taken from five to twenty trees in each of ten plots located in five widely separated areas in the southern part of the state. Two disks $\frac{1}{2}$ inch in diameter (0.20 sq. in. or 0.99 sq. cm. in area) were cut simultaneously, one from each half of the leaf, by a specially devised and constructed leaf punch (fig. 1). As soon as the desired number of disks (50–200, usually 100) had been cut, they were emptied into aluminum weighing cans, placed in an oven at 100° C. for 1 hour, and then dried to constant weight in a mechanically ventilated oven at 65° C. The disks from each chamber of the punch were placed in separate weighing cans. Values reported in the table are thus the means of duplicate samples taken simultaneously from one lot of leaves.

These tests were of shorter duration than the juice-quality tests; comparisons could therefore be made between oil-sprayed and nonsprayed leaves. The 1–2% light-medium or medium grades of petroleum-oil sprays used in these tests were applied at different times of year, and leaf-disk samples were taken at intervals from sprayed and nonsprayed trees after each application.

A rather limited number of tests was made to determine whether the addition of 2,4-dichlorophenoxyacetic acid (2,4-D) to the oil would tend to increase or nullify the effect of the oil on dry matter in the leaves. These tests were made in conjunction with other leaf dry-matter tests. The amount of 2,4-D used was usu-

ally 8 p.p.m., based on total volume of spray.

Results

JUICE-QUALITY TESTS.—Tabulated results pertain primarily to the effects of the three insecticides—DDT, parathion, and petroleum oil—on the total soluble solids, total acids (as citric), ratio of soluble solids to acids, and the ascorbic acid in orange and grapefruit juice. Data in tables are grouped in such a way that the proper comparison of companion lots is possible. For example, in table 1 five DDT values (tests 3–7) should be compared with one oil value (test 8); in table 4 one parathion value (test 13) should be compared with four oil values (tests 14–17).

Although in some instances the differences are not very great, data in table 1 show that, with one exception (test 22), concentrations of total soluble solids were higher in DDT-sprayed fruit than in oil-sprayed fruit. The mean concentration of soluble solids in the DDT-sprayed fruit was 12.69%, and in the oil-sprayed, 12.00%. The difference between these means was found to be significant at the 5% level.

Differences in concentrations of total acids in DDT-sprayed and oil-sprayed fruits were less than for the soluble solids. The means of the acid concentrations for the two treatments were very similar—1.15% for the DDT-sprayed and 1.11% for the oil-sprayed. Although the difference between these means is very little, twenty of the thirty-three tests (61%) showed the DDT-sprayed fruit to have a higher total acid content than the oil-sprayed. There was also very little difference between the ratios of soluble solids to acids as a result of the two treatments (11.2:1 and 11.1:1, respectively).

In a supplementary test to determine possible effects of successive applications of DDT on juice quality of Valencia oranges, two plots of twenty trees each

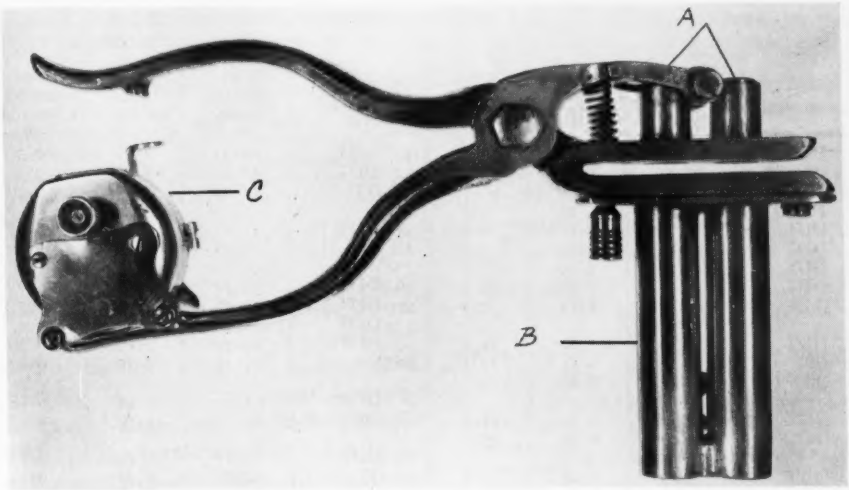

FIG. 1.—Leaf punch. *A*, twin disk cutter; *B*, detachable disk receivers; *C*, counter. There is a ¼-inch hole in the bottom of each disc receiver.

were sprayed in the fall of the year with 1¾% medium emulsive oil. During the following year, one of these plots was treated at intervals of 1–2 months, a total of eight times, with DDT; the other plot received no further treatment. Fruit samples from each plot were tested 1 month after the last application of DDT. Results of these tests are shown in table 2. Although differences in concentration of constituents of the juice of fruit from the two plots were not great, they definitely show that repeated applications of DDT did not cause a decrease in soluble solids and acids in the juice.

In connection with these findings reference is made here to the results of CULLINAN (8), who found, among other things, that DDT applied to a light type of soil at the rate of 25 pounds per acre retarded the growth of cantaloupes, cucumbers, tomatoes, and several other

TABLE 1

RELATIVE EFFECTS OF DDT AND PETROLEUM-OIL SPRAYS ON ORANGE JUICE

Test no. and treatment*	% total soluble solids	% total acids (as citric)	Ratio (soluble solids/acids)	Test no. and treatment*	% total soluble solids	% total acids (as citric)	Ratio (soluble solids/acids)
1. DDT	11.91	1.20	9.9	31. DDT	12.51	1.22	10.3
2. Oil	11.31	1.19	9.5	32. DDT	11.92	1.20	9.9
				33. Oil	11.30	1.19	9.5
3. DDT	11.91	1.21	9.8				
4. DDT	11.51	1.26	9.1	34. DDT	11.23	1.36	8.3
5. DDT	11.03	1.26	8.8	35. Oil	10.51	1.34	7.8
6. DDT	11.23	1.29	8.7				
7. DDT	11.71	1.23	9.5	36. DDT	12.72	1.27	10.0
8. Oil	10.43	1.12	9.3	37. DDT	12.78	1.19	10.7
				38. DDT	12.92	1.31	9.9
9. DDT	13.38	1.00	13.4	39. DDT	12.72	1.24	10.3
10. Oil	12.84	0.85	15.1	40. Oil	12.58	1.30	9.7
11. Oil	11.96	0.85	14.1				
				41. DDT	13.78	1.45	9.5
12. DDT	13.12	0.89	14.7	42. Oil	12.72	1.50	8.5
13. Oil	11.91	0.79	15.1				
				43. DDT	12.23	1.40	8.7
14. DDT	13.32	1.04	12.8	44. Oil	12.06	1.32	9.1
15. DDT	13.78	1.03	13.4				
16. Oil	12.92	1.00	12.9	45. DDT	11.96	1.12	10.7
				46. Oil	11.91	1.12	10.6
17. DDT	14.97	1.14	13.1				
18. DDT	14.44	1.12	12.9	47. DDT	13.44	1.38	9.7
19. DDT	15.07	1.09	13.8	48. Oil	13.02	1.17	11.1
20. DDT	14.44	1.15	12.6				
21. Oil	13.78	1.15	12.0	49. DDT	13.12	1.17	11.2
				50. Oil	12.51	1.15	10.9
22. DDT	13.18	0.95	13.9				
23. Oil	13.18	1.02	12.9	51. DDT	13.78	1.15	12.0
				52. Oil	12.51	1.26	9.9
24. DDT	12.84	0.97	13.2				
25. Oil	11.91	1.06	11.2	53. DDT	10.57	1.26	8.4
				54. Oil	10.03	1.32	7.6
26. DDT	13.12	0.93	14.1				
27. Oil	11.91	0.86	13.9	Mean, DDT	12.69†	1.15	11.2
				Mean, oil	12.00†	1.11	11.1
28. DDT	11.23	0.75	15.0				
29. DDT	10.77	0.86	12.5				
30. Oil	10.63	0.80	13.3				

* DDT used in kerosene and Velsicol AR-60 or tetralin.

† Difference between means significant at 5% level.

vegetables. Attention is also called to the more recent work of FOSTER (9), who found that DDT persisted in the soil and was toxic to certain varieties of beans, rye, and oats after 4 years.

For another supplementary test made to determine the relative effects of oil spray and HCN on quality of grapefruit juice, three plots were selected in a grapefruit grove in the Coachella Valley. Plot 1 was sprayed with oil in December, 1946, and again in January, 1947. This plot was also fumigated with HCN soon after each spraying. Plot 2 was sprayed with oil once in December, 1946, and was fumigated twice, at the same time as plot 1. Plot 3 was not sprayed with oil but was fumigated twice, at the same time as plots 1 and 2. Light-medium oil (2%) and a 24-cc. dosage of HCN (7) were used. Results of juice tests, made on May 2, 1947, are shown in table 3. They show that the oil spray caused a decrease in the concentration of total soluble solids in grapefruit juice as in orange juice. Concentration of the juice of grapefruit from plot 3 was 20.8% greater than that of the fruit from plot 1. Here again, as with the orange juice, the effect of the oil on the concentration of total acids was not so decisive. As might be expected, variation in total acids caused variation in the ratios.

Data in table 4 show that, except in two tests (22 and 42), the concentrations of total soluble solids in parathion-sprayed fruit, as in DDT-sprayed fruit, were higher than in their companion lots of oil-sprayed fruit. Mean concentration for the parathion-sprayed fruit was 12.46 %; for the oil-sprayed fruit, 11.73%. The difference between these means was significant at the 5% level.

Although the difference between the mean concentration of total acids in the parathion-sprayed fruit (1.24%) and in the oil-sprayed fruit (1.17%), as shown in table 4, was a little greater than that shown in table 1 for DDT and oil, there were three tests (22, 36, and 42) in which the acid concentrations were lower in parathion-sprayed fruit, and two paired tests (7 and 8, and 9 and 10) in which the values were the same for both treatments. (Note that three of the low parathion values shown in table 4—tests 3, 4, and 5—are compared with the value for nontreated, not oil-sprayed, fruit.) The ratios of soluble solids to acids were variable, but the means (10.4:1) were the same for both treatments. HARDING (11), working with early Florida oranges, found that parathion-treated fruit contained a higher concentration of soluble solids than nontreated fruit. On the other hand, this treatment did not appear to

TABLE 2

EFFECT OF SUCCESSIVE APPLICATIONS OF DDT SPRAY ON JUICE OF VALENCIA ORANGES

Plot no. and treatment	% total soluble solids	% total acids (as citric)	Ratio (soluble solids/acids)
1. Oil only.....	12.01	1.2	10.0
2. Oil+8 applications of DDT.	12.50	1.2	10.4

TABLE 3

RELATIVE EFFECTS OF OIL SPRAY AND HCN FUMIGATION ON GRAPEFRUIT JUICE

Plot no. and treatment	% total soluble solids	% total acids (as citric)	Ratio (soluble solids/acids)
1. Oil-sprayed twice; fumigated twice.	7.50	1.17	6.4
2. Oil-sprayed once; fumigated twice.	8.03	1.37	5.9
3. Fumigated twice.	9.06	1.36	6.7

affect the concentration of total acids materially.

ASCORBIC ACID TESTS.—No tests were made to determine the effects of parathion on ascorbic acid content of orange juice, but the data show (table 5) that, in their respective test groups, the juice from the oil-sprayed fruit contained considerably less ascorbic acid than that from the nontreated, the no. 899-treated, and the DDT-treated fruit. The mean of the concentrations of ascorbic acid in the juice of oil-sprayed fruits was 57.28 mg/100 ml, and the mean for all others was 62.99 mg/100 ml, a difference of 5.71 mg/100 ml.

LEAF DRY-MATTER TESTS.—The results of the dry-matter tests for navel orange leaves are shown in table 6, and those for Valencia orange leaves in table 7.

In a limited number of tests, possibly not enough to compensate for sample variation, results indicated that 2,4-D, when added to the oil, tended to nullify the depressing effect of the oil alone on the amount of dry matter in the leaves (table 8).

Apparently, navel leaves were less responsive than Valencia leaves to the depressing effect of the oil, or they were more responsive to the effect of the 2,4-D. (Data in tables 6 and 7 indicate that

TABLE 4

RELATIVE EFFECTS OF PARATHION AND PETROLEUM-OIL SPRAYS ON ORANGE JUICE

Test no. and treatment	% total soluble solids	% total acids (as citric)	Ratio (soluble solids/acids)	Test no. and treatment	% total soluble solids	% total acids (as citric)	Ratio (soluble solids/acids)
1. Parathion	11.37	1.51	7.5	24. Parathion	13.78	1.06	13.0
2. Oil	11.11	1.38	8.1	25. Parathion	13.78	1.09	12.6
				26. Oil	11.63	0.94	12.4
3. Parathion	11.23	1.24	9.1				
4. Parathion	12.11	1.29	9.4	27. Parathion	13.64	0.96	14.2
5. Parathion	12.01	1.31	9.2	28. Parathion	13.72	1.08	12.7
6. Nontreated	11.91	1.37	8.7	29. Oil	11.91	0.91	13.1
7. Parathion	13.22	1.02	13.0	30. Parathion	11.77	1.39	8.5
8. Oil	13.18	1.02	12.9	31. Oil	10.83	1.17	9.3
9. Parathion	13.02	1.06	12.3	32. Parathion	12.51	1.65	7.6
10. Oil	11.91	1.06	11.2	33. Oil	11.91	1.54	7.7
11. Parathion	13.92	1.04	13.4	34. Parathion	10.17	1.50	6.8
12. Oil	11.91	0.86	13.8	35. Oil	9.43	1.49	6.3
13. Parathion	13.98	1.33	10.5	36. Parathion	11.23	1.39	8.1
14. Oil*	11.91	1.06	11.2	37. Oil	10.37	1.41	7.4
15. Oil*	11.43	1.06	10.8				
16. Oil*	12.72	1.15	11.1	38. Parathion	11.31	1.36	8.3
17. Oil	13.01	1.17	11.1	39. Oil	10.03	1.32	7.6
18. Parathion	14.44	1.33	10.9	40. Parathion	13.32	1.29	10.3
19. Oil	12.51	1.15	10.9	41. Oil	12.51	1.14	11.0
20. Parathion	11.57	1.33	8.7	42. Parathion	11.91	0.78	15.3
21. Parathion	11.57	1.37	8.4	43. Oil	13.12	0.91	14.4
22. Parathion	10.91	1.15	9.5				
23. Oil	11.23	1.28	8.8	Mean, parathion	12.46†	1.24	10.4
				Mean, oil	11.73†	1.17	10.4

* Synthetic oil (OS 932 A-1). All other oils were the commonly used petroleum oils.

† Difference between means significant at 5% level.

the latter must be the true explanation.) Ten of the thirteen navel leaf samples sprayed with oil plus 2,4-D (table 8) had higher dry-matter values than those sprayed with oil alone, and four of the ten had higher values than the controls. Although four of the Valencia leaf samples also had dry-matter values that were higher than those of the controls, only six of the fourteen samples sprayed with oil plus 2,4-D had higher dry-matter values than those sprayed with oil alone.

Although these tests were relatively few, results are in line with those of the preceding dry-matter tests (tables 6, 7), which indicate that the commonly used petroleum oil has a depressing effect on the amount of dry matter in citrus leaves and that at least in some of the tests the addition of 2,4-D to the oil has a tendency to nullify this effect.

TABLE 5

RELATIVE EFFECTS OF INSECTICIDES ON ASCORBIC ACID CONTENT OF ORANGE JUICE

Test no. and treatment*	Ascorbic acid (mg/100 ml of juice)	Test no. and treatment*	Ascorbic acid (mg/100 ml of juice)
1. None	67.39	14. DDT	60.07
2. Oil	64.46	15. DDT	64.46
		16. Oil	55.67
3. None	67.31	17. Oil	57.13
4. Oil	61.53	18. Oil	54.20
		19. Oil	55.67
5. No. 899†	57.13		
6. No. 899	57.13	20. DDT	65.93
7. Oil	48.31	21. DDT	64.46
		22. Oil	62.99
8. DDT	57.14		
9. DDT	54.21	23. DDT	76.18
10. DDT	61.53	24. DDT	65.93
11. DDT	61.53	25. DDT	64.46
12. DDT	60.07	26. DDT	65.93
13. Oil	49.81	27. Oil	62.99

* DDT used in kerosene and Velsicol AR-60 or tetralin.

† American Cyanamid Co.; di-2-ethylhexyl phthalate.

Discussion

Information was not always obtainable concerning the previous year's insecticide treatment of the groves in which the experimental plots were located. It was found, however, that trees in some of the plots had been sprayed with oil.

Results of previous tests (2) have shown that the tendency for oil sprays to cause a reduction in the concentration of soluble solids in the fruit juice may be carried over and have a similar though less marked effect on the juice of the im-

TABLE 6

RELATIVE PERCENTAGES OF DRY MATTER IN NONSPRAYED AND OIL-SPRAYED MATURE NAVEL ORANGE LEAVES, 1946

Test no.	Spray applied	Samples taken	% dry matter in leaves		
			Non-sprayed	Oil-sprayed	Difference
1	Feb. 18	Feb. 27	41.5	38.3	+3.2
2	Feb. 18	Mar. 12	41.3	38.7	+2.6
3	Feb. 18	Mar. 21	44.2	39.7	+4.5
4	Feb. 18	Mar. 26	43.4	40.3	+3.1
5	Feb. 18	Apr. 5	41.8	39.6	+2.2
6	Feb. 18	Apr. 16	37.7	36.9	+0.8
7	Feb. 18	July 3	40.7	40.2	+0.5
8	Feb. 18	Aug. 21	39.8	39.3	+0.5
9	Feb. 18	Oct. 3	40.1	39.8	+0.3
10	Feb. 20	Feb. 28	41.4	40.5	+0.9
11	Feb. 20	Mar. 13	42.4	41.0	+1.4
12	Feb. 20	Mar. 21	42.8	39.8	+3.0
13	Feb. 20	Mar. 29	40.9	42.4	−1.5
14	Feb. 20	Apr. 5	45.3	42.7	+2.6
15	Feb. 20	Apr. 12	46.0	41.6	+4.4
16	Feb. 20	Aug. 23	39.6	38.0	+1.6
17	May 14	May 23	43.0	40.2	+2.8
18	May 14	June 15	42.1	39.4	+2.7
19	May 14	July 10	41.2	40.3	+0.9
20	May 14	July 11	38.0	39.0	−1.0
21	May 14	Aug. 27	38.9	36.4	+2.5
22	June 7	July 2	39.1	39.1	0.0
23	June 7	Aug. 21	38.8	37.8	+1.0
24	June 7	Oct. 3	39.2	38.8	+0.4
25	Aug. 21	Oct. 3	39.6	37.7	+1.9
26	Aug. 21	Oct. 14	40.7	40.9	−0.2
Mean	.....	.....	41.1*	39.6*	+2.0 / −0.9

* Difference between these values is well above that required for significance at the 5% level.

mediately succeeding crop. Thus, it is possible that at least some of the juice and dry-matter values presented in the tables for fruit and leaves treated with oil, DDT, and parathion may be relatively low because the trees were sprayed with oil the preceding year, whereas others may be relatively high because the trees were *not* sprayed with oil the preceding year.

TABLE 7

RELATIVE PERCENTAGES OF DRY MATTER IN NONSPRAYED AND OIL-SPRAYED MATURE VALENCIA ORANGE LEAVES, 1946

Test no.	Spray applied	Samples taken	% dry matter in leaves		
			Non-sprayed	Oil-sprayed	Difference
1	Feb. 21	Feb. 27	46.0	43.3	+2.7
2	Feb. 21	Mar. 12	45.0	42.3	+2.7
3	Feb. 21	Mar. 21	46.6	42.7	+3.9
4	Feb. 21	Mar. 26	47.4	44.4	+3.0
5	Feb. 21	Apr. 5	48.3	46.7	+1.6
6	Feb. 21	Apr. 16	45.0	43.0	+2.0
7	Feb. 21	July 3	42.9	42.3	+0.6
8	Feb. 21	Aug. 21	41.4	40.6	+0.8
9	Feb. 21	Oct. 3	43.1	43.4	−0.3
10	June 5	July 1	42.0	43.3	−1.3
11	June 5	July 16	39.3	39.2	+0.1
12	June 5	Aug. 20	39.5	39.2	+0.3
13	June 5	Oct. 2	40.3	39.8	+0.5
14	June 6	July 2	42.4	42.9	−0.5
15	June 6	July 11	41.8	42.3	−0.5
16	June 6	Aug. 19	43.6	41.9	+1.7
17	June 6	Aug. 27	43.5	42.8	+0.7
18	June 6	Oct. 3	42.8	43.2	−0.4
19	June 6	Oct. 3	42.2	42.6	−0.4
20	Sept. 4	Oct. 2	41.0	41.2	−0.2
Mean			43.2*	42.4*	+1.6 / −0.5

* Difference not quite significant at the 5% level.

Other factors may have caused variations in the amounts of soluble solids in the juice of the fruit, and in the dry matter in the leaves, from the plots in different areas. BARR (1) found that application of oil or wax emulsions caused a significant reduction in photosynthetic activity, and hence a reduction in the concentration of carbohydrates, in leaves of maize. In view of the reduction in soluble solids in the fruit juice and in the dry matter in the leaves, as reported in our studies, a similar reduction in photosynthetic activity may occur in citrus when oil sprays are applied. If this is true, it seems probable that the intensity of such an effect in the citrus-growing areas of southern California would be influenced by the differences in climatic factors which prevail in the areas in which these experimental plots were located—coastal, inland, or intermediate.

The time of year that the insecticide is applied may have been another factor in variation. The juice-quality tests reported in the present paper were not planned to determine the possible relative effects of time of application of the insecticides, but such tests are now under way. THOMPSON and SITES (16) and SITES (15), in Florida, have shown that oil sprays applied to the Hamlin orange after August 1 caused a greater reduction in total soluble solids in the juice than if applied between June 1 and July 15. HARDING (11) reported similar results with "early oranges."

Variations in leaf dry-matter values may have been influenced also by the age of leaves. Although all leaves from which test samples were taken were full grown and of the same color, as nearly as could be detected visually, some leaves may have been 1 or even 2 years older than others. The insecticide may have had a more pronounced effect on young "mature" leaves than on old "mature" leaves, or vice versa. Other factors which may have caused variations in the values presented are the age of tree, rootstock, vigor of growth, devitalizing effect of insects, etc.

Any one or more of these factors in

variation may augment or reduce the effect of one insecticide more than that of another. The variations mentioned above refer not only to values for fruit and leaves from nontreated trees but also to variations in relative effects of the insecticides.

So far as known, the effects of DDT on the quality of citrus juice have not been previously reported. THOMPSON *et al.* (17, 18) quoted J. W. SITES, associate horticulturist in the Florida Citrus Experiment Station, as saying that no relation was found to exist between the time of application of parathion and the concentration of total soluble solids in the juice (unpublished data).

GRIFFITHS *et al.* (10) have just reported results causing them to conclude that parathion does not produce an off-flavor in fresh or canned Florida citrus juice.

In this series of experiments no attempt was made to determine the effects of parathion on the ascorbic acid content of orange juice. It is of interest, therefore, to note that, although HARDING (11) found a significantly higher concentration of soluble solids in parathion-treated fruit than in the controls, he found only a very slight increase in ascorbic acid—not enough to be of practical value.

TABLE 8

EFFECT OF 2,4-D IN OIL ON DRY MATTER IN NAVEL AND VALENCIA ORANGE LEAVES

KIND OF LEAVES	TOTAL NO. OF SAMPLES SPRAYED WITH OIL +2,4-D	NO. OF SAMPLES IN WHICH DRY-MATTER VALUES WERE:			
		Higher than in control samples	Lower than in control samples	Higher than in oil-sprayed samples	Lower than in oil-sprayed samples
Navel orange.....	13	4	9	10	3
Valencia orange...	14	4	10	6	8

Summary

1. Studies were made to determine the effects of certain insecticides—primarily petroleum oil, parathion, and DDT—on the quality of the juice in freshly picked oranges and on the dry matter in Valencia and navel orange leaves. A few tests were made on grapefruit juice.

2. Oil sprays caused a significant reduction in the concentration of total soluble solids in the juice of oranges and grapefruits and, in the majority of tests, in the dry matter of navel and Valencia orange leaves.

3. Parathion and DDT caused very little if any reduction in the concentration of total soluble solids in the juice of oranges.

4. The effects of the insecticides on the total acids in the juice, and hence on the ratio of solids to acids, were variable; but in about 60% of the tests the acids were at least slightly higher in fruits sprayed with parathion or with DDT than in those sprayed with oil.

5. The ascorbic acid content of the juice of the DDT-treated fruit was no-

ticeably higher than that of the oil-sprayed fruit.

6. The fact that oil sprays were usually found to cause a reduction in concentration of soluble solids and ascorbic acid in the juice should be of special interest to processors who are anxious not only to have a high concentration of soluble solids but also to have it as soon as possible after the picking season begins.

Grateful acknowledgments are made to Dr. WALTER EBELING and to Dr. W. H. EWART of the Division of Entomology, who furnished part of the fruit samples; and to Dr. W. B. SINCLAIR of the Division of Plant Physiology, who assisted in part of the analytical work.

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PERSISTENCE OF 2,4-D STIMULUS IN COTTON PLANTS WITH REFERENCE TO ITS TRANSMISSION TO THE SEED[1]

WAYNE J. MCILRATH, D. R. ERGLE, AND A. A. DUNLAP

Introduction

Information appears to be contradictory on persistence of the 2,4-D (2-4-dichlorophenoxyacetic acid) stimulus within the plant for an extended period of time after the initial application (**1**, **2**, **3**, **4**, **5**, **10**, **11**). EAMES (**4**, **5**) has stated that the 2,4-D stimulus is immediate and brief and that reports of it being long-continuing are based upon false interpretations. He has concluded (**4**): "The opinion that in many plants the stimulus of various growth-regulating substances continues for various periods of time is probably based on (*a*) the continuing activity of the abnormal meristems; and/or (*b*) the development, long after treatment, of the dormant buds injured (while growing) before dormancy. New tissues and organs formed after treatment are not affected." The work of DUNLAP (**3**), however, seems to indicate that the response of the cotton plant does not confirm this conclusion. Under certain conditions the stimulus persists for a considerable period of time as shown by the fact that seeds taken from bolls formed several weeks after the initial 2,4-D application produced malformed seedlings. BROWN *et al.* (**1**), on the other hand, found no evidence of carry-over of the 2,4-D stimulus in seeds collected in fields of cotton that exhibited heavy injury.

This investigation was therefore undertaken to amplify existing data on persistence of the 2,4-D stimulus within the cotton plant as indicated by injury to seed embryos formed prior to and at various time intervals subsequent to the initial application of 2,4-D.

Experimentation and results

EFFECTS OF RELATIVELY LARGE AMOUNTS OF 2,4-D

Stoneville 2B variety of American Upland cotton, grown one plant per 2-gallon jar of fertile Houston Black clay, was used in all phases of this investigation. In this experiment an attempt was made to determine the effect of large amounts of 2,4-D on (*a*) translocation of the stimulus into seed embryos which were formed prior to application (pretreatment bolls) and (*b*) its persistence within the plant as shown by injury to embryos subsequently formed (post-treatment bolls). For this purpose 60-day-old plants, grown outdoors and carrying bolls with a maximum age of 15 days, were divided into three treated series and one untreated control. Each plant in the treated series was sprayed with 5 ml. of an aqueous solution of the sodium salt equivalent to 0.1, 1.0, or 10.0 mg. of 2,4-D acid per plant. The spray was distributed as uniformly as possible over all the leaves, and extreme care was taken to prevent run-off. No spreader was added to the solutions.

All seeds produced were checked for malformation in resultant seedlings by germination in flats of sand which were kept moist with a complete nutrient solution. Severity of injury was classified

[1] Published with the approval of the Director of the Texas Agricultural Experiment Station as Technical Article no. 1349.

as one of three degrees: slight, moderate, or severe. Those seedlings assessed as having slight effects exhibited an abnormal vein pattern and slight malformation of the first true leaf (fig. 1). Moderate malformation was characterized by filiform leaf shape with fasciation of the veins at the midrib of the first true leaf and with a slight degree of malformation sometimes apparent in the second true leaf (fig. 1). Seedlings classed as having severe malformation were those which exhibited fusion of the cotyledons or

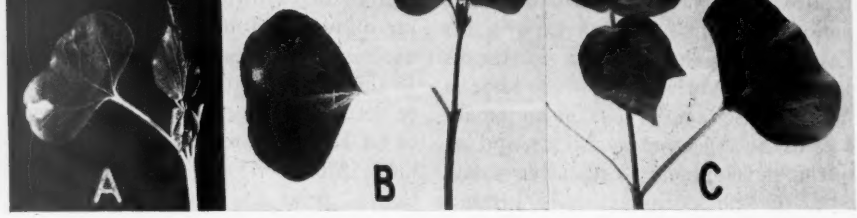

FIG. 1.—*A*, cotton seedling exhibiting slight malformation of first true leaf. *B*, seedling showing moderate malformation of first true leaf with slight modification of second. *C*, normal (control) seedling.

true leaves (fig. 2). Correlated with the leaf symptoms there was also a root response. Seedlings with a slight leaf malformation produced about as many lateral roots as the controls, but these roots were shorter and swollen. Seedlings with moderate or severe leaf symptoms produced very few or no lateral roots.

All young squares and flowers present on the parent plants at the time of spray applications were killed. In addition, the two higher amounts resulted in death of the leaves. All treatments caused swelling of the main stem and typical malformations in subsequently developed plant organs which increased in severity with increased amounts of 2,4-D applied (1, 3).

PRETREATMENT BOLLS.—The seedlings from seeds collected from bolls present on the plant at the time of spray applications (pretreatment bolls) in the 0.1-mg. and 1.0-mg. series all exhibited 2,4-D injury. Injury in the 0.1-mg. series varied from slight to severe, with 39.6% exhibiting moderate or severe symptoms. Germination of the seeds from bolls of the 0.1-mg. series was 96%, whereas that of seeds from bolls of the plants treated with 1.0 mg. was only about 35%, of which less than 30% survived beyond emergence of the radicle. This small survival of the latter series apparently was due primarily to the inability of these seedlings to develop lateral roots. All surviving seedlings displayed either moderate or severe leaf symptoms. Bolls of the 1.0-mg. series contained a large number of aborted seeds as contrasted with the 0.1-mg. series in which apparently all seeds matured. Pretreatment bolls of the 10.0-mg. group failed to develop and produce seeds. As compared with the controls, germination of seeds from pretreatment bolls was delayed about 4 days for the 0.1-mg. treatment and 8 days for the 1.0-mg. series. Once germination began in the treated series, however, it proceeded at a rate approximating that of the controls (9).

POST-TREATMENT BOLLS.—In all treatments vegetative growth of sprayed plants was suppressed for several weeks, and it was approximately 8 weeks after application before any new bolls were set. Seedlings produced from seeds collected from these bolls showed 78.3% malformation in the 0.1-mg. series, 46.7% in the 1.0-mg. treatment, and 52.0% in the 10.0-mg. group. Moderate or severe leaf symptoms were exhibited by 20.8% of the seedlings in the 0.1-mg. series. No severe leaf symptoms on seedlings were observed for the other two treatments. The higher percentage of seedlings from the 0.1-mg. group exhibiting malformations is probably to be accounted for by a higher percentage of the applied 2,4-D having entered the plant. At the higher dosages a large percentage of that applied was probably lost through death of leaf tissues (**8**).

Seeds taken from bolls formed 14 weeks after the initial application did not produce malformed seedlings in any series. Therefore, in this experimental set of plants the 2,4-D stimulus, as shown by its carry-over into seed embryos, was retained in the plants for a minimum period of at least 8 weeks, but such retention did not exceed 14 weeks.

INFLUENCE OF DEVELOPMENTAL STAGE ON TRANSMISSION OF 2,4-D STIMULUS TO SEED

Results of the foregoing experiment indicated that dosage and plant developmental stage at time of application both influenced the extent to which the 2,4-D stimulus was transmitted to the developing embryos. Therefore, in the second experiment smaller amounts of 2,4-D were applied at three stages prior to boll formation in the plants' developmental cycle: namely, seedling, floral primordia, and anthesis. Plants were sprayed in the seedling stage when the first two small true leaves were evident, at the floral primordia phase when the first-formed floral primordia had floral bracts (squares) about 1 cm. long, and at anthesis upon the opening of the first flower. Greenhouse-grown plants in this experiment were treated in the manner previously indicated with 5 ml. (2 ml. in seedling stage) of an aqueous solution of the sodium salt of 2,4-D in acid-equivalent quantities of 0.01 or 0.04 mg. per plant. Grasselli spreader was added to these solutions at the rate of 2 ml. per liter.

FIG. 2.—Cotton seedling produced from seed of pretreatment boll of 1.0-mg. series showing fusion of first true leaves (*arrow*). Original apical meristem of seedling completely embedded inside of tissue of fused leaves preventing additional stem growth for several weeks. Photograph taken 4 months after seed germination.

Of the plants treated at the three

stages, only those sprayed at anthesis showed a marked carry-over of the 2,4-D stimulus into the seed embryos subsequently produced. Seeds from plants treated at either the seedling or floral primordia stage yielded seedlings showing a very small percentage of 2,4-D injury (table 1), all of which was of a slight nature. The significance of these small percentage values is further reduced by the fact that 1.6% of the seedlings from seeds of the untreated plants exhibited leaf characteristics very similar to that indicated as a slight malformation for the treated plants.

In the seedling series the amounts of 2,4-D applied were sufficient to cause extreme malformation of main stem leaves subsequently developed and loss of apical dominance by the main stem apex. Renewed growth took place through development of vegetative lateral branches. In view of the long time between 2,4-D application and the development of the first bolls (table 1), it appears likely that the stimulus was almost exhausted in inducing malformations of leaves formed on the main stem and any that remained affected the vegetative lateral growth. Therefore, there was little or no movement of the 2,4-D stimulus into the developing embryos. An observation in support of this assumption was that all leaves of the vegetative laterals beyond their fourth or fifth node were normal (fig. 3).

The amounts of 2,4-D applied at the floral primordia stage were not lethal to the apical meristems but instead stimulated main stem elongation in the 0.01-mg. series and the development of vegetative laterals in the 0.04-mg. group (fig. 4) (**7**). With this stimulated vegetative growth it is quite possible that large quantities of carbohydrates were translocated into these active vegetative meristems at the expense of the developing floral primordia. Inasmuch as it has been shown that the 2,4-D stimulus moves with translocatable carbohydrates (**8, 12**), it seems feasible that the stimulus was concentrated and effective largely in these active vegetative meristematic tissues rather than moving into the existing floral primordia. It is also well to point out that, of the small percentage of seed injury observed when plants were treated at this stage with 0.1 mg. of 2,4-D, over 80% of it was observed in seeds from bolls set on fruiting branches initiated on main stem growth produced after application. The difference in seed carry-over

TABLE 1

EFFECT OF DEVELOPMENTAL STAGE OF COTTON PLANT ON TRANSMISSION OF 2,4-D STIMULUS TO SEED EMBRYOS

No. of seedlings observed	Developmental stage when 2,4-D was applied	Milligrams of 2,4-D applied	Percentage of seedlings exhibiting leaf malformation	Weeks after treatment to formation of:	
				First boll	Last boll
472	Seedling	0.01	2.1	16	21
79	Seedling	.04	0.0	21	22
292	Floral primordia	.01	3.3	3	7
208	Floral primordia	.04	1.9	9	17
258	Anthesis	.01	23.3	<1	5
289	Anthesis	0.04	63.0	<1	5

of the stimulus between the 0.01-mg. and 0.04-mg. applications at this stage, if it is significant, is to be accounted for by the longer time interval after application to the formation of bolls in case of the latter. With this longer interval of time, more of the stimulus was exhausted in meristematic tissues of the numerous floral primordia, and thus a concentrating of the 2,4-D stimulus occurred at these points. The stimulus was not entirely exhausted in the fully developed flowers present on the plant at the time of treatment, however, for injury was

FIG. 3.—Cotton plant (116 days old), treated with 0.01 mg. 2,4-D acid-equivalent at seedling stage, showing development of two vegetative lateral branches.

inducing malformations of vegetative growth.

Plants treated at anthesis, in contrast to those at the floral primordia stage, were not stimulated in their vegetative growth (main stem or vegetative laterals) by the 2,4-D applications (fig. 4). Consequently, the principal movement of carbohydrates in these plants was to the also found in seed embryos formed over a 5-week period following 2,4-D application (table 1). In the 0.01-mg. treatment the percentage of embryos affected and the severity of the effect were uniformly distributed over the ensuing 5 weeks. Of the seedlings showing leaf malformations, 19.6% of this number were of the moderate type and the remainder slight.

In the plants treated with 0.04 mg. there was a slightly greater concentration of the stimulus in the embryos formed within the first $2\frac{1}{2}$ weeks subsequent to 2,4-D application as evidenced by symptoms of seedlings produced from seeds of these bolls (table 2). There was also a higher rate of moderate and severe leaf symptoms exhibited by seedlings produced from seeds formed within this period.

FIG. 4.—Cotton plants (116 days old), treated at (*A*) seedling, (*B*), floral primordia, and (*C*) anthesis with 0.04 mg. 2,4-D acid-equivalent; (*D*) untreated plant. Note numerous vegetative lateral branches on plant treated at floral primordia stage.

At the end of the 5-week period the plants treated at anthesis had attained their full boll load, and no more bolls were set during the course of the experiment (6). Consequently, it was impossible to determine how much longer the stimulus would have been effective on the developing embryos had the setting of bolls continued.

Discussion

It is evident from the results that the 2,4-D stimulus continues over a considerable period of time in the cotton plant and is manifested by injury to seed embryos formed after the initial 2,4-D application. The period of time over which the effects of this 2,4-D stimulus will continue to appear in the embryos is probably controlled in large measure by the extent of vegetative growth (and carbohydrate metabolism associated therewith) subsequent to application and prior to or concurrent with the floral phase of development. That is, if vegetative growth occurs over a relatively long time interval after 2,4-D application and prior to seed formation, little or no injury will result to the embryo. Under such circumstances it appears that the stimulus is exhausted in inducing malformations of vegetative growth. Further, it appears that if vegetative growth is stimulated by the 2,4-D treatment, even though the time interval between application and fruiting is relatively short, most of the stimulus will be exhausted in the vegetative meristems with

little carried over to the embryos. In those cases where little or no vegetative growth takes place in the interim between application and fruiting, however, the stimulus may persist within the plant resulting in injury to the developing embryos over a period of several weeks (in excess of 8 weeks in some cases).

TABLE 2

PERSISTENCE OF 2,4-D STIMULUS WITHIN COTTON PLANT AS SHOWN BY TRANSMISSION OF STIMULUS TO SEED EMBRYOS FROM 3 TO 35 DAYS AFTER APPLICATION

Days between application and boll initiation	Percentage of resultant seedlings exhibiting 2,4-D injury			
	Slight	Moderate	Severe	Total
3	66.7	33.3	0.0	100.0
4	60.0	0.0	0.0	60.0
5	38.1	14.3	0.0	52.4
7	47.6	19.1	0.0	66.7
9	57.1	0.0	0.0	57.1
11	56.0	8.0	8.0	72.0
12	63.2	10.5	0.0	73.7
13	31.3	37.5	0.0	68.8
16	9.1	9.1	36.3	54.5
18	64.7	0.0	0.0	64.7
3–18	49.7	11.4	3.6	64.7
19–35	54.1	4.1	2.5	60.7

Summary

1. Sixty-day-old cotton plants were treated with the sodium salt of 2,4-D (2,4-dichlorophenoxyacetic acid) in acid-equivalent amounts of 0.1, 1.0, or 10.0 mg. to determine the effect of large amounts of 2,4-D on (*a*) translocation of the stimulus into seed embryos formed prior to application and (*b*) its persistence within the plant as shown by injury to embryos subsequently formed. Cotton plants at the developmental stages of seedling, floral primordia, and anthesis were also treated with 2,4-D acid-equivalent amounts of 0.01 and 0.04 mg. to determine the effect of plant age on the transmission of the 2,4-D stimulus into seeds developed subsequent to the application.

2. All seeds from bolls (1–15 days old) set prior to treatment with 0.1 mg. or 1.0 mg. produced seedlings which exhibited symptoms of 2,4-D injury. Bolls in the 10.0-mg. series failed to develop.

3. Some seedlings from seeds produced in bolls initiated 8 weeks after application in the 0.1-, 1.0-, and 10.0-mg. series exhibited 2,4-D injury. Seeds formed 14 weeks after application did not yield malformed seedlings in any series.

4. Plants treated with 0.01 or 0.04 mg. of 2,4-D at the seedling or floral primordia stage showed no significant transmission of the stimulus into the seed embryos.

5. Seed embryos formed in bolls initiated in a 5-week period subsequent to application at the anthesis stage showed significant 2,4-D injury.

6. A discussion is presented of some factors determining whether cotton seed embryos formed subsequent to application will be injured by the 2,4-D stimulus.

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MESQUITE SEED AND SEEDLING RESPONSE TO 2,4-D AND 2,4,5-T

BYRON O. BLAIR[1]

Introduction

This paper presents the results of studies on germinating seeds and young seedlings of mesquite, *Prosopis juliflora* var. *velutina* (Woot.) Sarg., a serious noxious range plant in the southwestern United States. The points investigated were: (*a*) the response of mesquite to certain organic herbicides in comparison with other plants already tested, (*b*) the comparative toxicity of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) to mesquite seeds and seedlings, and (*c*) the response of seeds and seedlings tested in this manner in comparison with that of mature trees tested with foliage sprays of 2,4-D and 2,4,5-T.

Material and methods

The bio-assay Petri-dish method (**2**) for determining low concentrations of 2,4-D in aqueous solutions was used. In the tests of germinating seeds, three replicate groups of seeds were used in each concentration. Scarified seeds were germinated in $4\frac{1}{2}$-inch Petri dishes to which had been added 10 ml. of the herbicidal solution to be tested. Reagents tested were free acid of 2,4,5-T and 2,4-D at concentrations of 1, 2, 5, 10, and 50 p.p.m. Three dishes containing distilled water were used as controls. Dishes were placed at random in an incubation oven. Rotating and changing the positions of the dishes daily compensated for temperature variations in the oven. Germination for 72 hours at 26–27° C. produced suitable growth for measurement.

Seedling studies were conducted in the same manner as described in testing seeds. Seeds were germinated for 24 hours on a blotter in distilled water, and the resulting seedlings were selected for uniformity and transferred to Petri dishes containing the herbicidal solutions to be tested.

Growth was measured in three ways: length of root, length of hypocotyl, and

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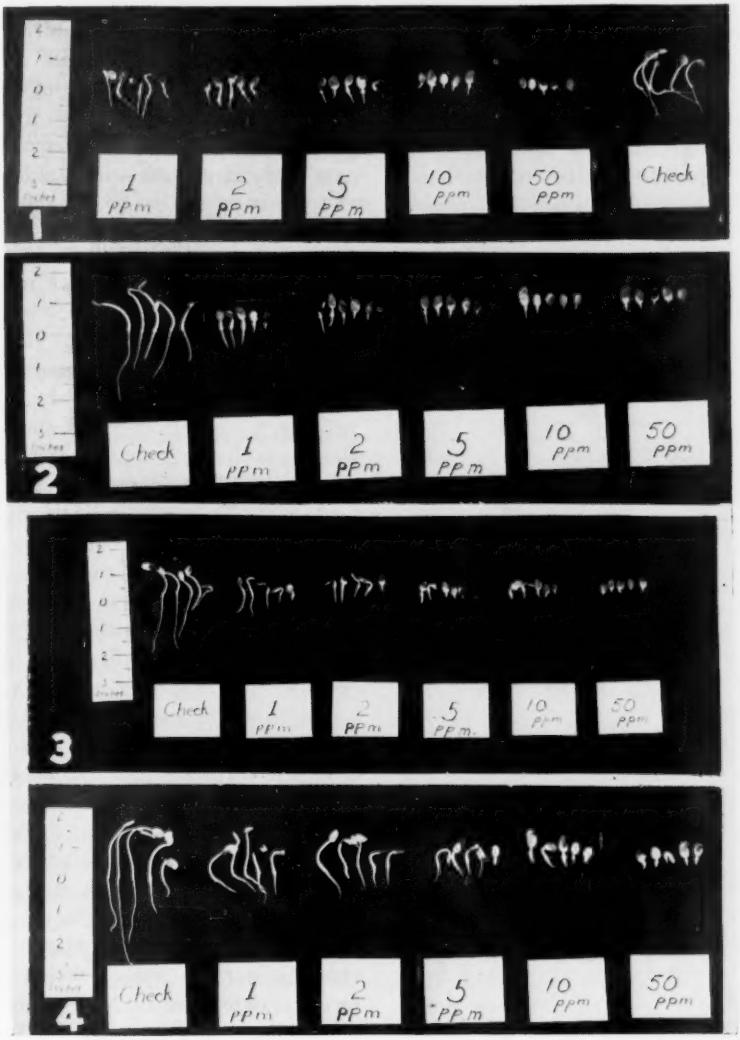
total fresh weight (dried by blotting but without heat). Since change in fresh weight reflects principally total water intake, it provides a good indication of physiological activity. Effect of herbicidal treatment was expressed as percentage inhibition in growth of treated seeds and seedlings in comparison with controls.

Results

In tests on seeds, germination and growth of seedlings were markedly inhibited by both 2,4-D and 2,4,5-T, even



FIGS. 1–4.—Suppression effect of 2,4,5-T (figs. 1, 3) and 2,4-D (figs. 2, 4) on mesquite seeds (figs. 1, 2) and seedlings (figs. 3, 4). Note thickening of hypocotyl at root-stem transition zone in treated seedlings.

in a concentration of only 1 p.p.m. (figs. 1, 2). Suppression by 2,4-D at this level was greater than by 2,4,5-T according to all three criteria measured (table 1). Between 1 and 10 p.p.m. suppression differences were slight, with 2,4,5-T comparatively more effective in the higher concentrations. Suppression of root and hypocotyl growth at 10 p.p.m. for both 2,4-D and 2,4,5-T was above 90%. Concentrations of 50 p.p.m. produced almost total suppression, so that root and hypocotyl growth was too limited for accurate measurement. Suppression as measured by fresh weight was much less but showed a trend similar to measurements for hypocotyl and root growth.

In tests on seedlings suppression by 2,4-D and 2,4,5-T, although in general not so great as on seeds, was definite at 1 p.p.m. (figs. 3, 4). Hypocotyl growth at this concentration showed 2,4,5-T to be more effective in inhibition than 2,4-D. Concentrations above 1 p.p.m. showed 2,4,5-T to be more effective in suppressing both root and hypocotyl growth. Wet-weight measurements indicate a slight growth stimulus by 2,4-D at 1 p.p.m.

TABLE 1

PERCENTAGE INHIBITION OF MESQUITE SEEDS AND SEEDLINGS BY 2,4-D AND 2,4,5-T BASED ON CONTROLS IN DISTILLED WATER

CONCENTRATION* (P.P.M.)	GERMINATING SEEDS		24-HOUR-OLD SEEDLINGS	
	2,4-D	2,4,5-T	2,4-D	2,4,5-T
	Root length			
1	70.2	58.3	61.0	63.3
2	74.7	72.8	62.3	72.8
5	88.0	89.4	83.4	95.7
10	90.2	94.0	86.5	99.4
	Hypocotyl length			
1	88.0	77.6	61.8	75.0
2	86.7	89.1	63.8	83.2
5	96.7	97.7	89.7	99.0
10	98.7	98.9	92.4	99.5
	Fresh weight†			
1	30.8	18.0	0.3‡	19.9
2	31.5	23.4	1.2	25.3
5	36.4	34.0	33.6	38.6
10	35.6	50.9	37.3	33.7

* Growth was insufficient at 50 p.p.m. to measure accurately by all three criteria.

† Seedlings dried by blotting but not subjected to heat prior to weighing.

‡ Percentage stimulation in this one case.

Discussion

The results indicate that mesquite, while not so sensitive as some other plants (1), is susceptible to the herbicides tested. The suppression of mesquite seeds and young seedlings when treated with even 1 p.p.m. of 2,4-D and 2,4,5-T shows conclusively that in early growth stages the plant is responsive to these herbicides. This may indicate that failure of herbicidal concentrations to kill mature trees of this species is due to lack of sufficient absorption and translocation rather than to inherent resistance to these compounds. Differences in suppression rate in general show 2,4,5-T to be more toxic to young plants than 2,4-D. This is in agreement with results of foliage spray tests on mature trees.

Total fresh weight could serve as a valuable measure of herbicidal inhibition. A considerable amount of time is required to make and compute measurements of root and hypocotyl growth, but such measurements are paralleled by changes in fresh weight which can be rapidly determined. Measurement of herbicidal inhibition by fresh-weight criteria would be time-saving and effective in screening many herbicides.

Summary

1. Seeds and seedlings of mesquite, *Prosopis juliflora* var. *velutina* (Woot.) Sarg., were cultured for 72 hours in aqueous solutions of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) at concentrations of 1, 2, 5, 10, and 50 p.p.m. Suppression was measured and expressed in root and hypocotyl growth, and total fresh weight, in comparison with controls.

2. Seeds and young seedlings of mesquite were found to be responsive to 2,4-D and 2,4,5-T but not so sensitive as some other plants. Concentrations above 1 p.p.m. show 2,4,5-T to be more toxic to mesquite than is 2,4-D. This has also been found true of foliage sprays applied to mature trees.

3. Suppression of both seeds and seedlings by 1 p.p.m. of either herbicide shows the sensitivity of young plants to these compounds. Applications of much higher concentrations to mature plants as foliage sprays have not resulted in kills, indicating either lack of absorption and translocation or greater resistance of mature plants to these compounds.

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THE TOMATO AS A TEST PLANT FOR GROWTH-REGULATORS

WENDELL R. MULLISON

Numerous methods for testing growth-regulating activities of chemical compounds have been developed. The *Avena* coleoptile and pea tests require precise techniques and carefully controlled environmental conditions. Other methods have been used which are less exacting. References to literature dealing with some of these other methods may be found in publications by BROWN and WEINTRAUB (1), FISCHER and YOUNG (2), HITCHCOCK (3), HITCHCOCK and ZIMMERMAN (4), and KING (5).

In a screening program involving the testing of many chemicals several considerations are important. First, the test method should be sensitive enough so that a response can be detected, yet not so sensitive as to be unselective. Second, the test should be easy and rapid to perform so that a large number of tests may be run in a given time period. Third, the method should be usable throughout the year. Fourth, the test should be one which can be readily duplicated by other workers.

The following techniques are believed to satisfy these requirements. They are of two types: (*a*) a plant-immersion and (*b*) a single-drop technique. Although both methods may be used for preliminary testing of compounds for activity and later for evaluating the degree of activity of selected materials, in our laboratory the single-drop method is used mainly for evaluation. These tests

are elaborations of the tomato technique used at Boyce Thompson Institute (**6**). There the test material was applied to the plant either as a lanolin paste or in a spray or was mixed with the soil; cuttings were treated by dusting or soaking in solutions. In the present work the upper part of the stem and its attached leaves are dipped into a composition containing the material to be tested. This chemical is formulated, usually as an aqueous solution or emulsion, at concentrations of 0.12 gm. and 0.36 gm. per 100 ml. (1 lb. and 3 lb. per 100 gal.). The formulations are poured into unwaxed paper cups[1] 4 inches in diameter and $2\frac{3}{8}$ inches deep. These cups are used because they are cheap and disposable. The latter feature is time- and labor-saving and eliminates contamination when active materials are used. Young tomato plants grown in soil in 4-inch clay pots until $3\frac{1}{2}$–4 inches tall are used. A plant is inverted and immersed in the paper cup containing the formulation until the tip touches the cup bottom, then immediately removed and set upright for the excess to drip off on a drainboard, then placed in a greenhouse. After 24 hours observations are made for epinastic responses and for injuries which may be brought about by contact action of the chemical being tested. Ten days later observations are made for formative and systemic effects. Materials meriting further investigation are tested in an evaluation program.

The single-drop technique has been used both for injecting material into the plant and for applying it to the foliage. The ease and rapidity of the latter method of application has resulted in its being more often used. A drop, 0.006 ml. of solution or emulsion, is placed on a young tomato leaf of a plant about 4 inches tall with a micrometer-driven syringe. This is a 1-cc. tuberculin syringe with an attached half-inch no. 27 needle,[2] and the plunger is controlled by a micrometer head. The plunger head has been ground flat to provide good contact with the driving face of the micrometer screw. The knurled headed bushing in which the micrometer head is fastened unscrews from the brass housing which supports the needle (fig. 1). Advancing the micrometer screw 0.015 inch delivers a 0.006-ml. drop in the particular apparatus now in use. The basic idea has been used in several widely divergent fields such as paper chromatography, insect and animal physiology, and micro-manipulative techniques. This idea has been modified in our laboratory to its present form. It is an excellent instrument to use in the single-drop bean or tomato test as well as for injection purposes.

Weight of treated tomato plants has been found to be unsatisfactory as a criterion of activity of the compounds being tested. Weight measurements sometimes indicate the growth inhibition brought about by the material under test. Many times, however, this is not the case as plants with pronounced formative effects may weigh as much as the controls. Because of such findings growth responses of the tomato in these tests have been divided into six classes, to each of which has been assigned an arbitrary numerical value. After the compound is tested, each plant is classified according to degree of response

[1] No. 492-SE $\frac{3}{4}$-pint heavy-duty ice-cream and food Pac-Kups made by Dixie Cup Company, Easton, Pennsylvania.

[2] B-D Yale tuberculin syringe no. 1 YT and B-D Yale no. 27, $\frac{1}{2}$-inch stainless steel needle. Becton, Dickinson and Company, Rutherford, New Jersey.

shown. Figures for all plants in a particular test are averaged, thus allowing assignment of a numerical value to the potency of any particular chemical used. This facilitates comparisons among the activities of different chemicals. The classes and values assigned to them are:

Class	Value	Growth response
0	0	No effect
1	15	Epinasty and leaf modification with no stem swelling
2	30	A few irregular stem swellings and sometimes a coalescence of several such areas at the base of the plant
3	50	General stem swelling either completely around the stem and extending over two internodes or swollen areas scattered along the entire stem; stems often bent
4	80	Stems swollen from tip to base and usually markedly bent; stunting; sometimes cracked stems and necrotic areas; little apical growth
5	100	Plants dead

In general, each class repeats the symptoms of the categories preceding it.

Examples are given to illustrate this system, and results are shown (table 1) for several common plant growth-regulating chemicals. In this test three tomato plants were used for each concentration. For purposes of comparison the activity of these compounds was also tested by the single-drop bean technique on cranberry beans.[3] Data in this experiment are the average of two replicates of ten bean plants each. A drop (0.006 ml.) of a 1200-p.p.m. solution (7 μgm.) was applied by means of the micrometer-driven syringe to one of the

[3] The light strain of cranberry beans (*Phaseolus vulgaris* var. Cranberry) used was obtained from the Michigan Bean Company at Saginaw, Michigan.

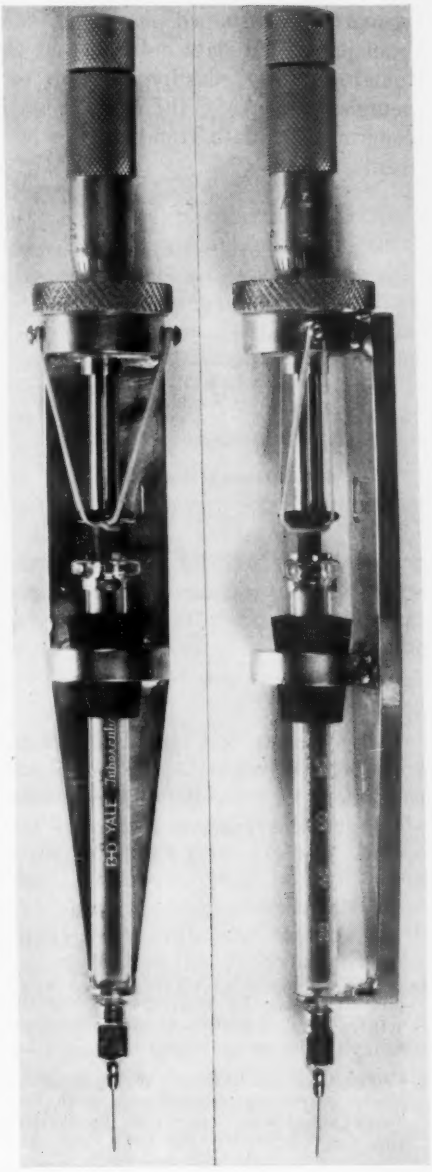

FIG. 1.—Front and side views of micrometer-driven syringe described in text.

primary heart-shaped leaves of each bean plant. The data indicate that the tomato is more sensitive to the compounds tested than the bean. This is confirmed by data from other experiments.

TABLE 1

Compound	Concentration (gm/100 ml)	Tomato (degree of plant response*)	Bean (repression in shoot growth shown by weight in gm.)
3-indoleacetic acid	0.12	15	21.2
3-indolebutyric acid	.12	15	19.5
	.36	50	.....
α-naphthaleneacetic acid	.12	87	17.5
	.36	93	.....
β-naphthoxyacetic acid	.12	50	22.0
	.36	80	.....
2-chlorophenoxyacetic acid	.12	30	19.2
	.36	50	.....
4-chlorophenoxyacetic acid	.12	80	8.3
	.36	100	.....
2,4-dichlorophenoxyacetic acid	.12	100	6.9
	0.36	100	.....
Control	0	0	20.1

* For derivation of values see text.

These tomato test techniques as described have proved to be quick and convenient for screening and evaluating plant growth-regulators of the 2, 4-D type.

Summary

1. A method is described using the tomato as a test plant for screening and evaluating plant growth-regulators.

2. A micrometer-driven syringe is described which greatly facilitates delivery of a number of small drops of uniform size.

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CHLOROPHYLL INHIBITION AND HERBICIDAL ACTION OF 3-(ALPHA-IMINO-ETHYL) 5-METHYL TETRONIC ACID[1, 2]

C. L. HAMNER AND H. B. TUKEY

Introduction

Certain coumarin derivatives inhibit seed germination and root and top growth of plants (**2, 3, 5, 6, 8**). This action has been associated with the unsaturated lactone ring which these compounds contain (**4**). Several other compounds having an unsaturated lactone ring also affect plant growth (**10**). Accordingly, preliminary trials were made with several tetronic acid derivatives, which are simple compounds having this structure (**1**). One of the most interesting was 3-(alpha-imino-ethyl) 5-methyl tetronic acid, reported upon in this paper.

Material and methods

The compound 3-(alpha-imino-ethyl) 5-methyl tetronic acid was tested on several plants, including White Banner wheat (*Triticum sativum*), Marketer cucumber (*Cucumis melo*), North Star hybrid sweet corn (*Zea mays*), Scarlet Globe radish (*Raphanus sativus*), a certified variety of Red Kidney bean (*Phaseolus vulgaris*), and a few other plants.

The methods of treatment were either to soak seeds in various concentrations of the chemical or to spray seedlings and plants with an aqueous solution. Seeds were soaked for 16 hours, generally at a concentration of 1000 p.p.m., or seedlings were sprayed at 1000 p.p.m. when they had reached a height of 2–6 inches. All work was done in the greenhouse, and all seeds were planted and seedlings grown in 4-inch pots containing equal amounts of sand, soil, and peat moss.

The first experiments were started on December 15, 1950. Others were conducted between December 15, 1950, and March 15, 1951. For the first experiments the chemical had been prepared by J. LECOCQ, École Polytechnique Institut, Paris, France (**9**). For later experiments the chemical was prepared by T. L. REBSTOCK, Michigan State College. Material prepared by REBSTOCK was considerably more active than that prepared by LECOCQ, perhaps because the latter material was several years old and partially oxidized.

Results

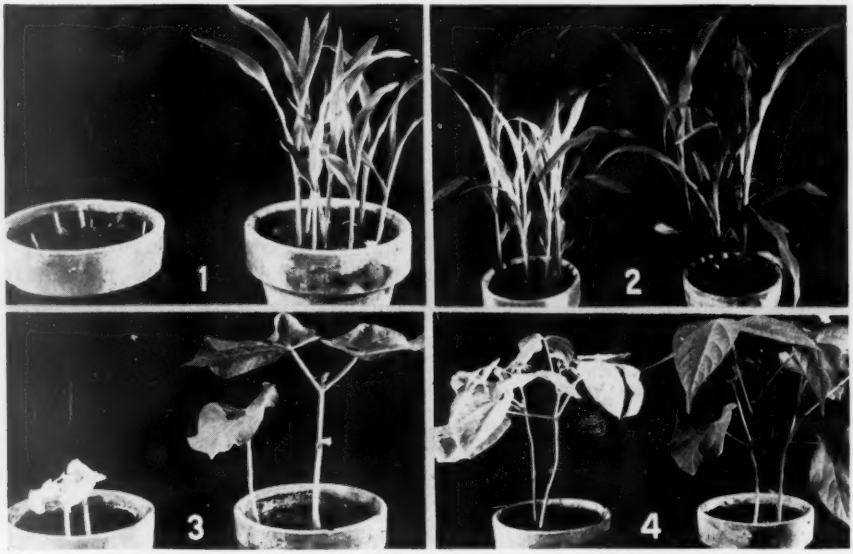
Corn was especially sensitive to the chemical. When grains were soaked in a solution at 1000 p.p.m. for 16 hours, seedlings which emerged were entirely lacking in chlorophyll and were inhibited in both root and top growth. Reserves in the grain were exhausted, and the plants eventually died (fig. 1). Although chlorophyll was completely lacking in treated seedlings, anthocyanin appeared not to be affected. When corn seedlings were sprayed with a similar solution, the leaves became straw-white in color several days after application and like tissue paper in texture (fig. 2). Both roots and tops were suppressed, and the plants died in 3–4 weeks.

When Red Kidney bean seeds were soaked 16 hours in the solution, 50%–90% of the emerging seedlings were com-

[1] This work was aided by a grant from the Rockefeller Foundation.

[2] Journal article No. 1240 of the Michigan Agricultural Experiment Station.

pletely lacking in chlorophyll. They were inhibited in both root and top growth and died shortly after emergence (fig. 3). The other 10%–50% of the plants grew normally for several days. However, shortly after the first heart-shaped leaves expanded, entire plants became chlorotic and eventually died (fig. 4). Older bean plants sprayed when they began to bloom gradually lost their green color, the loss of color proceeding from the margins of 5). Leaf blades frequently first showed a whitish area approximately 10–15 cm. from the sheaths. The blade became weakened at this point, resulting in its sharp downward collapse. Chlorosis then proceeded in both directions from the area of bending. Dissection of the terminal growing region showed absence of chlorophyll. The oldest leaves sometimes remained green for 10–14 days, but within 3 weeks the plants were dead.



FIGS. 1–4.—Effects of treatment with 3-(alpha-imino-ethyl) 5-methyl tetronic acid. Controls (*right*) and treated plants (*left*) in each picture. Fig. 1, corn, and fig. 3, bean seedlings, devoid of chlorophyll; from seed soaked in chemical. Fig. 2, corn, and fig. 4, bean seedlings, sprayed with compound; chlorophyll gradually disappears from leaves after spraying.

the leaves toward the midrib and from the tip of the blade toward the base. Pods frequently failed to fill and likewise became chlorotic. Root development was suppressed.

Wheat grains soaked in the solution for 16 hours produced seedlings which also emerged devoid of chlorophyll and shortly died. The kill was 100%. Young wheat seedlings sprayed with the solution became progressively chlorotic (fig.

In another experiment wheat and radish seeds were planted together. Ten days after emergence the seedlings were sprayed. Wheat and radish were both affected, but wheat more severely and more immediately than radish. The wheat became chlorotic and died within a week, whereas the radish plants slowly and gradually lost color, finally became white, and died.

Cucumber seeds which were soaked

for 16 hours in a solution at 1000 p.p.m. produced plants which were abnormal, inhibited in growth, and lacking in chlorophyll. When cucumber plants were sprayed, the older growth became mottled with whitish areas. New axillary growth was white and sharply stunted, giving the peculiar appearance of tiny rosettes of white appendages.

In an additional series of experiments several greenhouse plants were either sprayed or injected with a solution of the chemical. Tomato (*Lycopersicon esculentum*) seedlings 6 inches tall were sprayed at 1000 p.p.m. and developed stunted, chlorotic growth near the tips of the plants. Anthocyanin pigments, however, were not reduced. Snapdragon (*Antirrhinum majus*) seedlings which were similarly treated produced chlorotic growth in both new laterals and new terminals. Easter-lily plants (*Lilium longiflorum*) which were injected at the base with 1 ml. of the solution at 1000 p.p.m. just before the plants were coming into flower became chlorotic at the tips, and chlorophyll gradually disappeared from the clusters of flower buds. Coleus plants (*Coleus blumei*) similarly sprayed lost much of their chlorophyll, although the anthocyanin pigments were not affected. The coleus plants in consequence finally changed in color from red to a light lavender.

FIG. 5.—Gradual disappearance of chlorophyll from leaves of wheat seedlings sprayed with 3-(alpha-imino-ethyl) 5-methyl tetronic acid (*left*); untreated plants at right.

Discussion and conclusions

3-(Alpha-imino-ethyl) 5-methyl tetronic acid inhibits chlorophyll formation in plants. General plant development is suppressed, but otherwise no anomalous growth or formative effects are apparent. Food reserves are depleted, and the plant is exhausted and dies.

Because of the peculiar nature of its action the chemical may have value as a herbicide. No known herbicide is effec-

tive in completely killing deep-rooted perennials. Such materials as 2,4-dichlorophenoxyacetic acid (2,4-D), for example, may kill bindweed to a depth of 8–12 inches but may not result in killing the entire plant (7).

The compound is of special interest also because it appears to be extremely effective on monocotyledonous plants.

VAN OVERBEEK (11) has proposed that the mode of action of 2,4-D is related to coumarin. Attention is drawn to the fact that both coumarin and 3-(alpha-iminoethyl) 5-methyl tetronic acid have an unsaturated lactone structure.

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NUTRIENT SOLUTIONS FOR ORCHIDS

LEWIS KNUDSON

Introduction

This paper deals with a nutrient solution recently proposed by VACIN and WENT (8) for the germination of orchid seed and growth of the seedlings. Their solution, designated "Solution 5," is proposed as a replacement for KNUDSON'S solution C (5), since they stated that solution C is not well buffered. VACIN and WENT considered that a critical range of hydrogen-ion concentrations is essential for the germination of orchid seed and the subsequent growth of the seedlings. They stated (8): "KNUDSON as well as commercial growers expressed the opinion that the critical pH range . . . was between 4.5 and 5.5 for satisfactory germination." Such a statement I have never made and, if any commercial growers subscribe to that view, it has not come to my attention. In fact, in the article describing solution C it was specifically stated that for best growth the pH value of the culture medium should

be about pH 5.0. This refers to the pH following final autoclaving of the culture medium. As a matter of fact, in that particular paper best growth was noted when the initial pH was 4.35. This obviously is lower than the minimum pH considered by VACIN and WENT to be satisfactory for the germination of orchid seed.

In the course of experiments with my solution C, VACIN and WENT noted that the hydrogen-ion concentration of the culture medium increased with time and growth of the seedlings. In one experiment with *Epidendrum o'brienianum*, the pH value of the culture medium dropped from an initial pH of 5.46 to 3.78 after 190 days of growth, then to pH 3.12 after 277 days of growth. This led them to investigate the buffering capacity of solution C and the buffering effect of the individual salts. Having found that solution C was not well buffered, they developed a new nutrient solution designated as solution 5. It should be stated that no evidence was presented by VACIN and WENT that solution C was injurious at the lower pH value. It should be emphasized also that their solution 5 was proposed before they had tested its adequacy for the germination and growth of orchid seedlings.

To facilitate subsequent discussion, it is desirable to give here the formulas for both KNUDSON solution C and VACIN and WENT solution 5. They follow:

KNUDSON SOLUTION C

$Ca(NO_3)_2 \cdot 4H_2O$	1.000	gm.
$(NH_4)_2SO_4$	0.500	gm.
$MgSO_4 \cdot 7H_2O$	0.250	gm.
KH_2PO_4	0.250	gm.
$FeSO_4 \cdot 7H_2O$	0.025	gm.
$MnSO_4 \cdot 4H_2O$	0.0075	gm.
Sucrose	20.00	gm.
Agar	15.00	gm.
Distilled H_2O	1000.00	ml.

VACIN AND WENT SOLUTION 5

$Ca_3(PO_4)_2$	0.200	gm.
KNO_3	0.525	gm.
KH_2PO_4	0.250	gm.
$MgSO_4 \cdot 7H_2O$	0.250	gm.
$(NH_4)_2SO_4$	0.500	gm.
$Fe(C_4H_4O_6)_3 \cdot 2H_2O$	0.028	gm.
$MnSO_4 \cdot 4H_2O$	0.0075	gm.
Sucrose	20.00	gm.
Agar	16.00	gm.
Distilled H_2O	1000.00	ml.

It should be noted that solution 5 contains tricalcium phosphate. This salt is soluble only to the extent of 0.002–0.003 gm. in 100 ml. of water, and tricalcium phosphate is an extremely effective agent for the removal of heavy metals from solution. HOPKINS (**4**) precipitated tricalcium phosphate in his nutrient solution in order to remove iron and manganese. STEINBERG (**6**) used calcium carbonate for a similar purpose. By his method tricalcium phosphate is also precipitated and acts to adsorb heavy metals. Similar methods have been used by BAILEY and MCHARGUE (**2**), STOUT and ARNON (**7**), and others. It appeared probable that the use of tricalcium phosphate might result in chlorosis; that this occurs is clear from the experiments that follow.

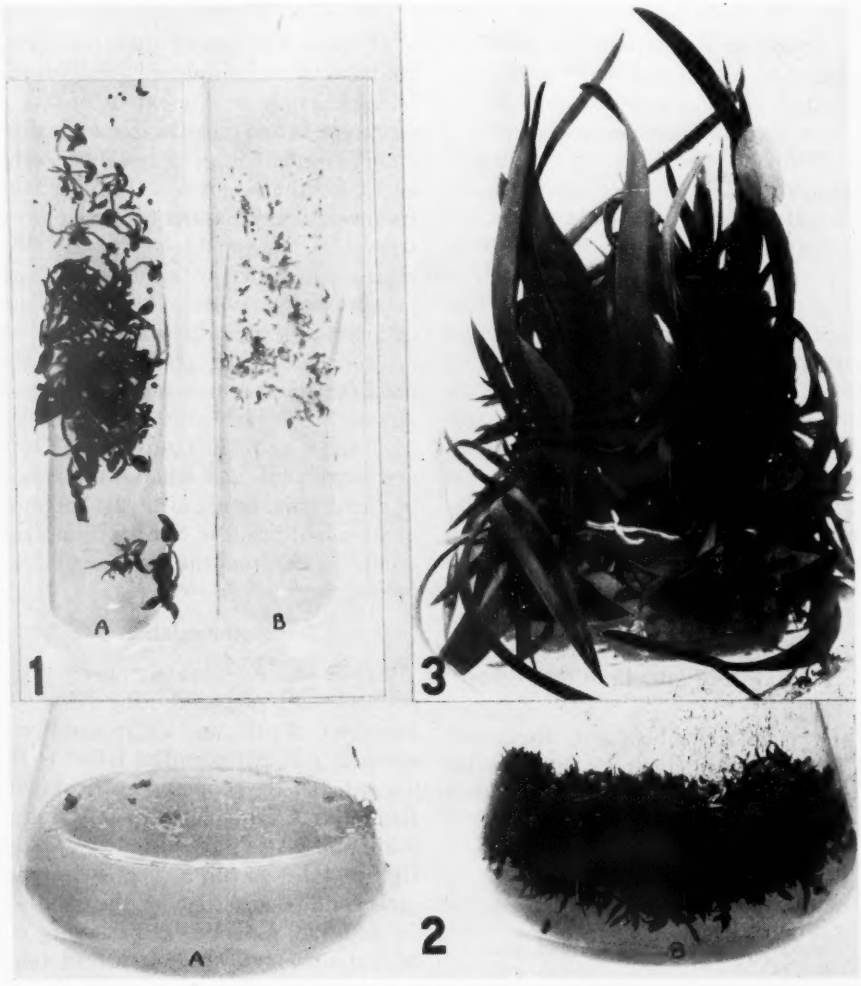
Experimentation

Experiment I.—On December 7, 1949, cultures were prepared using KNUDSON solution C at pH 5 and VACIN and WENT solution 5 at pH 5.7. The latter is the pH value of the proposed nutrient solution 5, but for comparative purposes solution 5 was also adjusted to pH 5.0. Culture tubes 20 cm. × 3 cm. were used, each containing 60 ml. of the solution. In addition, Erlenmeyer flasks of 500-ml. capacity were used, each containing 225 ml. of the culture medium. Hybrid *Cattleya* seed were used and seed of a species, *Cattleya skinneri*. They were freed of microörganisms by means of

calcium hypochlorite, and the techniques used during a period of many years were followed.

After 30 days germinating seeds were deep green in color on solution C, but on the VACIN and WENT solution the embryos were markedly chlorotic. Final data were obtained on November 24, 1950, the duration of the experiment being 352 days. Results are shown in table 1 and typical cultures of *C. skinneri* in figure 1.

The data reveal clearly that the VACIN and WENT solution 5 was inadequate



FIGS. 1–3.—Fig. 1, *Cattleya skinneri;* age 1 year. *A*, KNUDSON solution C, pH 4.90. *B*, VACIN and WENT solution 5, pH 5.0. Fig. 2, *Cattleya* hybrid; age 8 months. *A*, VACIN and WENT solution 5A, pH 5.4. *B*, KNUDSON solution C, pH 4.98. Fig. 3, *Cattleya* hybrid seedlings; age 33 months, on solution C. Initial pH 5.16; final pH 3.0.

for the growth of *Cattleya* seedlings. Seedlings on solution C had greater weight, approximately twenty times that of seedlings growing on solution 5 with an initial pH of 5.7 and approximately ten times greater when compared with seedlings on solution 5 with an initial pH of 5.0. It will be noted that the original pH value was not maintained in any solution. Seedlings on solution C at the conclusion of the experiment were deep green in color, producing roots, and were altogether thrifty.

It would appear that solution 5 was found unsatisfactory by VACIN and WENT, for, in a subsequent paper, VACIN (9) proposed a modification of solution 5. The new solution, designated "5A," is solution 5 to which has been added 5 ml. of an aqueous solution containing 10% hydrolyzed casein. Although this solution was not specifically proposed for seed of *Cattleya* but for seed of *Cymbidium*, it nevertheless appeared desirable to test this solution for *Cattleya* seed. VACIN stated (9): "The agar should be added and the pH adjusted to 5.5." This was done, and the results were again unsatisfactory. The experiment was begun on February 1, 1950, and the cultures photographed on September 28, 1950. Results are shown in figure 2.

That a change in the pH value following prolonged growth is without effect on *Cattleya* seedlings is revealed in figure 3. On March 15, 1947, very young seedlings were transferred from tube cultures to Erlenmeyer flasks containing solution C. The initial pH was 5.16. The culture was photographed 33 months later (fig. 3), and the pH value of the agar medium was determined and found to be pH 3. It is apparent that the seedlings were in a healthy state. The slight curling of the leaves is due to the fact that the agar medium had been reduced to 65 ml. from an original volume of 200 ml. Water in the culture medium was, therefore, close to unavailability.

Discussion

As indicated earlier, VACIN and WENT emphasized a critical range of hydrogen-ion concentration for the growth of orchid seedlings. This has no basis in fact. It is true that if the initial pH of the culture solution is below pH 4.5, *Cattleya* seed may not germinate, and certainly, at an initial pH below 4, seed of many genera may be killed. This is probably due to the increased availability of minor elements such as iron and manganese and in part due to the failure of absorption of calcium, as indicated by ARNON and JOHNSON (1). If, however, the initial pH value is close to 5, an increase in hydrogen-ion concentration with time is without any deleterious effect on the seedlings. It is probable that the seedlings adapt themselves to the higher hydrogen-ion concentration and that

TABLE 1

GROWTH OF "CATTLEYA" SEEDLINGS AND CHANGES IN PH WITH KNUDSON SOLUTION C AND WENT AND VACIN SOLUTION 5. (DURATION 352 DAYS)

Culture	Initial pH	Final pH	Fresh weight 100 seedlings (mg.)
	C. gigas × C. schroederae		
Solution C	4.90	3.63	460
Solution 5	5.70	4.59	20
Solution 5	5.00	4.37	40
	C. skinneri		
Solution C	4.90	3.41	1220
Solution 5	5.00	4.18	100

there is a gradual decrease in the content of such minor elements as iron and manganese owing to the continued absorption of these elements by the seedlings. This tolerance of high hydrogen-ion concentration has been repeatedly noted with *Cattleya*, *Cypripedium*, *Vanilla*, *Cymbidium*, *Oncidium*, *Phalaenopsis*, *Vanda*, and seedlings of other genera.

Solution 5 proposed by VACIN and WENT was based purely on theoretical grounds and on the assumption that the critical range of hydrogen-ion concentration is between pH 4.5 and 5.5. Solution 5A at the hydrogen-ion concentration proposed induces chlorosis and is not adequate for seedlings of *Cattleya* and undoubtedly not suitable for seedlings of other orchid genera. Furthermore, solution 5, contrary to the belief of VACIN and WENT, is not especially well buffered. There are indications that solution 5A might be suitable at pH 5. The addition of casein hydrolyzate is probably without value except as the amino acids may maintain iron in solution as indicated by the work of GABRIO and TISHKOFF (3).

Summary

1. VACIN and WENT (8) proposed a nutrient solution designated solution 5 as an improvement over KNUDSON solution C for the germination of orchid seed and growth of the seedlings. Experiments reveal that the VACIN and WENT solution 5 is unsuited for the purposes suggested. This is also true of the "improved" solution proposed by VACIN (9) and designated solution 5A.

2. It is indicated that the two solutions 5 and 5A are unsuitable because of the presence of tricalcium phosphate, which, at the pH values recommended, removes minor elements by adsorption, the result of which is chlorosis.

3. KNUDSON solution C is entirely adequate for the germination of seed of *Cattleya* and growth of seedlings.

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NEGATIVE RESULTS FOLLOWING EXPOSURE OF SEVERAL KINDS OF SEEDS TO COSMIC RAYS AND OTHER RADIATIONS AT HIGH ALTITUDES[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 627

Through the kindness of Professor MARCEL SCHEIN and certain of his graduate students in the Department of Physics, University of Chicago, it has been possible to send seeds of several species of angiosperms on flights to high altitudes in their cosmic-ray apparatus with the purpose of determining whether cosmic rays and other radiations might have mutagenic effects that would become evident in the seedlings developed from these seeds. In all cases the seeds were placed in thick-walled, screw-capped, glass bottles, with plastic caps screwed on tightly and firmly taped with several layers of transparent cellophane to make the seals airtight. The bottles were tied together and wrapped in metal foil to protect them against excessive heat in the transparent plastic-covered case which carried seeds and physical instruments.

The first seeds, consisting of *Lilium amabile* Palibin., *L. pumilum* DC., *L. regale* Wils., and *Brassica hirta* Moench, went up on November 18, 1947. The ascent began at 8:35 A.M. According to the flight data, the apparatus rose rapidly to an altitude of approximately 80,000 feet, following which it rose more slowly until it reached slightly more than 100,000 feet. At this altitude the seeds were subjected to a considerable intensity of heavy nuclei. The apparatus then descended rapidly and landed in Lake Erie near Toronto, Canada, at 4:30 P.M. Fortunately it became entangled in the net of some fishermen, who recovered it on November 21. It was returned to the University of Chicago on November 24, and the seeds were turned over to me.

During submergence in the waters of Lake Erie a part of the bottles leaked and allowed their seeds to become thoroughly soaked, but because of the cold temperature of the water no signs of germination were apparent. Generous numbers of seeds from each bottle were planted immediately in good loam soil in pots in the greenhouse. Rapid and almost complete germination occurred, with all seedlings appearing to be normal when compared with control seedlings growing alongside them.

The white mustard plants (*B. hirta*) were allowed to grow to maturity; they flowered and matured seeds during the winter of 1948. These seeds were in turn planted and mature plants obtained, but these were also like the controls in all obvious phenotypic characters.

The seedlings of the three species of *Lilium* were transplanted to the garden in the late spring of 1948, where they have been kept under observation since. The plants, however, have shown no visible phenotypic changes, appearing similar in all respects to the control plants of each of the species.

On March 9, 1948, seeds of the same species from the same sources as in the preceding flight were bottled, sealed, and handled just as in the former flight. A height of 60,000–65,000 feet was reached, with the apparatus remaining above 60,000 feet for a little over 1½ hours. These seeds were returned on March 12 and planted immediately. For some unknown reason, or reasons, not a single seed germinated.

Grains of Golden Bantam sweet corn and of Radium Brand spring rye were sent up on a flight on April 30, 1948, attaining an altitude of slightly less than 34,000 feet and remaining at approximately 30,000 feet for nearly 8 hours. When planted 2 days later, these seeds germinated as well as the controls, but again no phenotypic differences were observable when compared with controls, despite the fact that at the altitude reached these grains were near the maximum concentration of the giant atmospheric showers. The corn was self-pollinated, grains were produced, and a second generation was grown. These plants, however, showed no obvious phenotypic differences from their parents or from the controls grown from the original lot of grains.

A flight on October 13, 1948, carried seeds of Scarlet Globe radish and grains of Radium Brand spring rye to a height of approximately 65,000 feet, but no information on the length of exposure at this elevation was available. The seeds were returned and planted on October 16, and germination was closely similar to that of

[1] This work was supported in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

controls. Again these plants were apparently phenotypically like their controls.

With the exception of those seeds sent up on the second flight (none of which germinated) the seeds from the other experimental and the control lots showed approximately the same percentages of germination, and the growth and development of seedlings from them were essentially identical. Exposure to cosmic rays and other radiations, notably to the giant atmospheric showers at around 30,000 feet and to heavy nuclei at the 100,000-foot altitude, apparently resulted in no visible phenotypic alterations. Thus, under the conditions of these tests cosmic rays and other radiations seemed ineffective in producing cytogenetic effects on certain seeds and their seedlings.—J. M. BEAL, *Department of Botany, University of Chicago.*

DIMORPHIC PANICLE FORMATION IN POA PRATENSIS[1]

Progenies from approximately thirteen hundred accessions of *Poa pratensis* L. from various sources have been grown in the grass-breeding nurseries at Madison, Wisconsin. Among these was one sixteen-plant progeny that differed from the usual types in that panicles were produced at two levels. The upper level was carried terminally upon the plant culms in the regular manner, these culms exceeding the vegetative leaf mass by approximately one-third to one-half of the total plant height. The lower level of panicles was characterized by being carried upon short culms that generally approximated the natural height of the leaves as the plants stood in the field. They seldom exceeded the natural leaf height and were usually interspersed within the leaf mass (fig. 1). Although this dimorphic habit of panicle development may occur more frequently in some grasses, it apparently is extremely rare in Kentucky bluegrass.

FIG. 1.—Member of progeny from plant 645-8. Note the lower-story panicles borne upon short culms approximating height of leaves.

It is the purpose of this note to call attention to this departure from the usual type of panicle formation and to present data regarding the performances of progenies derived from seed of the two levels of panicles.

Material and methods

All the plants developed from the original accession produced two levels of panicles. The particular seed lot was taken from a natural stand of this species growing near Mount Vernon, Washington, where it was collected by Mr. E. J. KREISINGER. Approximately 37% of the plants grown the first generation were aberrant or off-type, thus indicating that the parent was only moderately apomictic in reproductive habits.

Individual panicles from the two levels were harvested separately from seven of the sixteen plants in the original progeny. Seeds of these were subsequently germinated in small pans containing sterilized compost. When seedlings had attained a height of approximately 2 inches, they were reset individually in plant bands and subsequently space-planted in the field.

During their first fruiting year they were examined for dimorphic panicle habit and for the frequency of aberrant or off-type plants within

[1] Results of co-operative work between the Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, U.S.D.A., and the Wisconsin Agricultural Experiment Station, Madison.

the progenies. Two different sets of progenies were grown, these being mature for examination during the summers of 1945 and 1949, respectively. The data have been combined in table 1, however, since they were reasonably similar during the two years.

Herbarium specimens of representative plants of the first generation tested were prepared. These are on file in the writers' laboratory.

Results

The data compiled for the second-generation progeny tests are summarized in table 1. These are for individual progenies of the seven plants tested. They are in good agreement both for the expression of the dimorphic panicle habit and for the frequency of off-type plants.

Some variation occurred with respect to the number of panicles in the lowermost level. Generally, however, the plants were essentially similar in the frequency of plants having two levels of panicles. The difference of this frequency was significant ($P < .05$) in one case, namely, in plant 645-16. No difference was found ($P > .05$) when the entire population from the two sources was compared. Explicitly 88.5% of the plants derived from terminal panicles produced panicles at both levels compared with 93.3% of those from lower-level panicles. Approximately 91% of all the plants grown and examined exhibited the dimorphic habit.

The variations that occurred in the frequencies of off-type plants were not significant, either when the individual progenies were considered or when all the populations of plants from the two panicle levels were compared. Approximately 28% and 33% of the plants in progenies from terminal and lower-level panicles, respectively, were of divergent growth form from the prevailing plant types. The average frequency of off-type plants in the total population was 30.5%, which compared satisfactorily with the 37.5% observed in the first generation. This difference is likewise not statistically significant.

TABLE 1

COMPARISONS OF PROGENIES FROM TERMINAL AND LOWER-LEVEL PANICLES FOR FREQUENCIES OF DIMORPHIC PLANTS AND PROPORTION OF SEXUAL REPRODUCTION

Parent	From terminal panicles			From lower-level panicles		
	No. plants grown	No. plants dimorphic	No. plants off-type	No. plants grown	No. plants dimorphic	No. plants off-type
645-1	17	15	5	15	14	5
645-2	17	14	4	17	16	6
645-3	36	33	17	38	33	19
645-8	14	14	1	20	19	6
645-10	41	39	8	38	37	11
645-15	42	39	10	37	34	7
645-16	41	30	14	43	41	14
Total	208	184	59	208	194	68
Percentage	.....	88.5	28.4	.....	93.3	32.7

Summary

Plants of a single-panicle progeny of Kentucky bluegrass (*Poa pratensis* L.) were dimorphic in panicle formation, one level being produced terminally upon the plants and the second or lower level among the leaves. Second-generation progenies indicate that this is a genetic characteristic of this particular morphological type. With the exception of dimorphic panicle habit, plants studied were similar in other respects to types not uncommon in the species. —E. L. NIELSEN[2] *and* D. C. SMITH,[3] *Madison, Wisconsin.*

[2] Agronomist, Division of Forage Crops and Diseases, U.S. Department of Agriculture.

[3] Professor of Agronomy, Wisconsin Agricultural Experiment Station, Madison.

CURRENT LITERATURE

The Piperaceae of Northern South America. By WILLIAM TRELEASE and TRUMAN G. YUNCKER. Urbana, Ill.: University of Illinois Press, 1950. Vol. 1, pp. 1–434, figs. 1–393; vol. 2, pp. 435–838, figs. 394–674. $10.00.

The rich plant life of the American tropics is far from being well known. Although every expedition yields new collections, distributional data, and species previously undescribed, much remains to be done before all available material and information have been brought together and integrated. An excellent example of this type of detailed systematic study dealing with an important family is provided by *The Piperaceae of Northern South America.*

Included in this work are a brief introduction by the junior author, complete descriptions of 840 species of Piperaceae (Pepper family) as found in the Guianas, Venezuela, Colombia, and Ecuador, artificial keys for their identification, 674 full-page figures, all of which are photographs of types of new species and other critical specimens, a bibliography (pp. 754–755), an index of collections cited (pp. 756–816), and an index of names (pp. 817–838). The extensive collections used in the preparation of this monograph were obtained on loan from the major herbaria in this country and abroad.

At the time of his death in 1945 the scientific legacy of the late Professor TRELEASE included numerous notes on Piperaceae taken in European herbaria and many unpublished descriptions of new entities. It is indeed fortunate that the University of Illinois secured the services of Professor YUNCKER as research professor in botany for the academic year of 1946–1947 for the purpose of collating this unpublished material and preparing it for publication. How well the junior author has carried out his task can best be judged from the following figures. Of the 454 species of *Piper* treated in this work, 201 new species are described by both authors and 45 by the junior author, as are 8 new varieties against the 61 new varieties, 14 new combinations, and 4 new names credited to TRELEASE and YUNCKER. Of the 365 species treated under the other large genus, *Peperomia*, 116 new species are proposed by both authors and 37 by the junior author, while 20 new varieties are described by TRELEASE and YUNCKER and 10 new ones by YUNCKER. In addition, 11 new combinations and 2 new names are proposed in this genus by both authors. The four remaining genera are small compared with *Piper* and *Peperomia*. *Ottonia* has 1 species, as does *Sarcorhachis*. *Pothomorphe* has 2 species, under which 3 new combinations are proposed, and *Trianaeopiper* has 17 species, 6 of which are described as new, together with 3 new varieties. The junior author prepared the artificial keys and, assisted by his wife, photographed all specimens reproduced in the figures. It is indeed gratifying to see that within 5 years following the death of Professor TRELEASE his unfinished work was completed, prepared for publication, and published in such beautiful style.

The reader will be struck by the fact that a large number of the specimens listed was collected in the last 25 years or so, mostly by American botanists. Thus the age of exploration has not yet come to an end, as more new species are bound to be described from the tropics as soon as intensified collecting is possible in heretofore poorly accessible regions. Yet this monograph will long remain the standard source of reference for this group of plants as found in the area designated and beyond it.—THEODOR JUST, *Chicago Natural History Museum, Chicago 5.*

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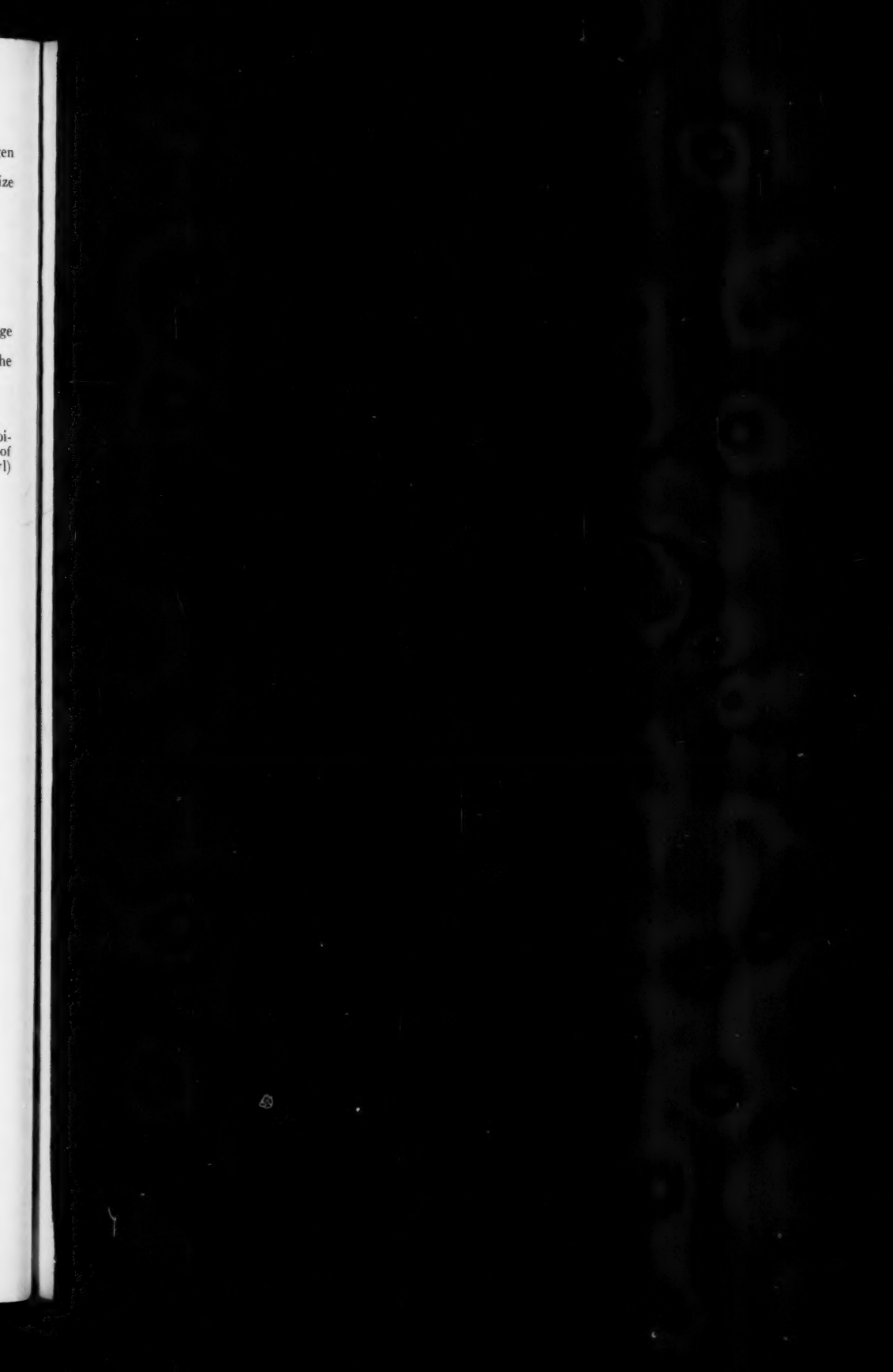
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